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Insights resulting from quantitative bioanalysis in studies of drugs and driving

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Samenvatting

In deze studie onderzochten we de waarde van kwantitatieve bioanalyse van drugs in studies over rijden onder invloed van drugs. Gebaseerd op het feit dat rijden en het gebruik van psychoactieve substanties niet samen gaan, zou men kunnen stellen dat rapportage van kwantitatieve resultaten niet nodig is, vooral voor routine analyses voor rijden onder invloed van drugs, en dat het volstaat om de aanwezigheid of afwezigheid van een drug (klasse) te bepalen om de prevalentie van rijden onder invloed van drugs te bestuderen of een schatting van het ongevallen-risico te maken. Het voorbeeld van alcohol, waar het risico exponentieel toeneemt met toenemende bloed alcohol concentraties (waardoor het risico op ongevallen onderschat wordt bij het gebruik van een lage grenswaarde), suggereert dat het gebruik van kwantitatieve bepalingen van drug concentraties in biologische vloeistoffen een toegevoegde waarde zou kunnen hebben.

In de Belgische wetgeving omtrent 'rijden onder invloed van drugs' zijn zowel speeksel- als bloedmonsters opgenomen met gelijkaardige grenswaarden. Het effect van het gebruik van deze gelijke grenswaarden in gepaarde monsters van speeksel en bloed in de algemene rijdende bevolking werd onderzocht aan de hand van een dataset van ongeveer 3000 gepaarde monsters. Kwantitatieve analyse toonde aan dat 2,6 keer meer bestuurders positief waren bij confirmatie-analyse in speeksel dan in bloed.

Hoewel de wijziging van de Belgische verkeerswet (invoering van zowel screening als confirmatie in speeksel) in voege ging op 1 oktober 2010, wordt de bevestigingsanalyse nog steeds uitgevoerd op bloedmonsters, maar met lagere grenswaarden dan voorheen. Om de invloed van deze gewijzigde handhavingsprocedure op het aantal vals positieve screenings te beoordelen, werden twee datasets van elk ongeveer 4000 positieve screenings vergeleken. Kwantitatieve analyse toonde aan dat minder valse positieve screenings werden waargenomen sinds de implementatie van de nieuwe wetgeving en dat meer recent drugsgebruik wordt aangepakt. Het totaal aantal positieve screenings is gedaald, maar dit is uitsluitend te wijten aan het lagere aantal positieve screenings voor cannabis, hetgeen kan worden verklaard door het veel langere detectievenster voor THCCOOH in urine (vroegere wetgeving) dan voor THC in speeksel (huidige wetgeving).

De meeste risico-inschattingen zijn berekend op basis van de nominale indeling (positief of negatief) van de 'cases' en 'controles'. Ook de concentratie die gevonden wordt in de biologische matrices kan van belang zijn. We vergeleken de verdeling van plasmaconcentraties van meerdere psychoactieve stoffen tussen een representatieve steekproef van alle bestuurders (n = 2750) en zwaargewonde bestuurders (n = 377). Kwantitatieve analyse toonde aan dat hogere amfetamine en benzoylecgonine concentraties werden gevonden bij gekwetste bestuurders. Daarnaast werd ook een trend voor hogere concentraties voor benzodiazepines en Z-drugs aangetoond. Ongevallen-risico's zouden daarom ook moeten bepaald worden met betrekking tot de concentraties van de psychoactieve substanties en niet enkel op basis van de aan- of afwezigheid van een substantie in een biologisch staal.

Zelfrapportage is de meest gebruikte methode voor de prevalentie-meting van het gebruik van cannabis. In de Belgische roadside studie werd zowel een vragenlijst als de resultaten van bioanalyse verzameld. De overeenkomst tussen de zelfrapportage en de resultaten van bioanalyse werden onderzocht aan de hand van gegevens van 2949 respondenten. Uit kwantitatieve analyse bleek dat de zelfgerapporteerde gegevens het gebruik van cannabis onderschatten en dat deze onderschatting het duidelijkst was bij recente gebruikers.

Naast het feit dat er bij de wetgeving rond rijden onder invloed van drugs kwantitatieve bioanalyse van de concentratie van illegale drugs nodig is, speelt bioanalyse ook een zeer belangrijke rol in het epidemiologisch onderzoek naar druggebruik en gerelateerd ongevallenrisico.

Summary

In this study we examined the value of quantitative bioanalysis of drugs in studies on drugs and driving. Based on the fact that the use of psychoactive substances should not be combined with driving, one could argue that, especially for routine testing for driving under influence of drugs, reporting quantitative results is not necessary and that it is sufficient to determine the presence or absence of a drug (class) to study the prevalence of drugged driving or to estimate the crash risk. The example of alcohol, where crash risk is increasing exponentially with increasing blood alcohol concentrations (hence using a low cut-off for the presence of alcohol underestimates crash risk) suggests that using quantitative drug concentrations in bio-fluids might have added value.

In the Belgian DUID-legislation both oral fluid and blood samples are taken and analysed with similar cut-offs. An objective of this thesis was to investigate the effect of using these similar cut-offs in paired samples of oral fluid and blood in a population of general drivers. For this purpose a dataset of almost 3000 paired samples was investigated. Quantitative analysis showed that 2.6 times more drivers confirmed positive in oral fluid compared to blood.

Although the Belgian traffic law amendment (introducing both oral fluid screening and confirmation) went into force on October 1st, 2010, confirmation is still performed on blood samples but with lower cut-offs than before. To assess the influence of this change in enforcement procedure on the number of false positive screenings, two datasets of approximately 4000 positive screening cases each were compared. Quantitative analysis disclosed that fewer false positive screenings were observed since the implementation of the new legislation and that more recent drug use was targeted. The total number of positive screenings has dropped with the new legislation, but this is solely due to a lower number of positive screenings for cannabis, which can be explained by the much longer detection window of THCCOOH in urine (previous legislation) than THC in oral fluid (current legislation).

Most risk estimations are calculated based on nominal categorisation (positive or negative) of the cases and controls. The concentration found in the biological matrices can also be of interest. We compared the distribution of plasma concentrations of several psychoactive substances between the general driving population (n= 2750) and seriously injured drivers (n= 377). Quantitative analysis illustrated that higher amphetamine and benzoylecgonine concentrations were found in injured drivers. In addition, a trend towards higher concentrations of benzodiazepines and Z-drugs was also observed. Accident risks should therefore also be assessed in relation to substance concentrations not only the presence or absence of a drug in a biological sample.

Self-reporting is the most widely used method to measure prevalence of use of psychoactive substances. In the Belgian roadside study both questionnaire data and results of bioanalysis were collected. An objective of this thesis was to investigate the consistency between self-report and results of bioanalysis. Data on 2949 respondents providing questionnaire data and the results of bioanalysis of blood and/or oral fluid samples were investigated. Quantitative analysis revealed that the self-reported data underestimated the use of cannabis and that this underestimation was most obvious for recent use.

Besides the fact that the DUID legislation requires quantitative bioanalysis of drug concentrations, quantitative bioanalysis is also of significant importance in epidemiological research on drug use and in research on accident risks associated with psychoactive substances.

CHAPTER 1

GENERAL INTRODUCTION

What is the state of the art in drugs and driving? What was the background of the EU-funded DRUID project, and what were the major outcomes of the project? What is the current state of legislation on driving under the influence of alcohol, drugs and medicines in Belgium and other European countries?

Chapter 1 describes the background of the thesis, the research questions and the outline of the dissertation.

Introduction

Recent statistics reveal that almost 25 700 people died on European roads in 2014 and more than 200 000 people were seriously (life-changing) injured. [1] For every death on Europe's roads there are an estimated four permanently disabling injuries such as damage to the brain or spinal cord, eight serious and fifty minor injuries. Approximately a quarter is estimated to be linked to drink-driving. [2] Although alcohol is the most prevalent and well-documented psychoactive substance affecting drivers, the role of illicit or medicinal drugs in driver impairment and traffic accidents has been a cause for concern and an object of research for several decades. [2,3]

The use of psychoactive substances can influence injury risk in several ways: the state of mind of drivers can be influenced; driving performance tasks such as keeping the right track and reaction time can be negatively influenced which may result in a higher crash risk. In addition, research has shown that drivers under the influence of drugs and/or alcohol seem to be using seatbelts less frequently and are found to be speeding more than sober drivers. [4–6]

Results of experimental studies indicate that several illicit drugs can have an influence on driving performance, some with dose-dependent effects. Cognitive and/or psychomotor impairment are observed with chronic use of illicit drugs, causing impairment of driving performance even when intoxication is no longer present. [7] In addition, some therapeutic drugs such as benzodiazepines, antihistamines and antidepressants have shown to be impairing in experimental studies. [7]

Driving under influence of a combination of alcohol and drugs is not uncommon. International prevalence studies have found a high number of combinations in drivers involved in a traffic accident. [7] The prevalence of alcohol-drug combinations ranged from two to almost twelve percent, combinations of different drug classes were observed in three to more than nine percent of injured drivers. [3]

In this dissertation the term 'drugs' refers to psychoactive substances that include the illicit (drugs of abuse) and licit (psychoactive medication) drugs.

1. Epidemiological studies on drugs and driving

1.1. Methods to determine the relationship between drug use, driving impairment and traffic crashes[3]

There are two different methods to study driving under influence of drugs, namely experimental and epidemiological studies.

In **experimental studies**, drugs are administered in different doses to volunteers; the impairing effects are measured and compared to administration of a placebo or a positive control (for instance alcohol). Assessment of psychomotor and cognitive functions, tests in a driving simulator or 'real' driving tests are used to evaluate the performance of the volunteers. Disadvantages of this type of study are the use of drug doses that are often much lower than those used 'in the street' and the fact

that the small sample sizes and a multitude of variable factors make it difficult to compare or combine results of different studies.

Epidemiological studies examine the prevalence of drugs in various driving populations. Examples are: roadside surveys, prevalence studies in subpopulations (such as injured and killed drivers), accident risk studies, responsibility analyses, interview-surveys and pharmaco-epidemiological studies. The choice of survey is subject to different issues like legislation, data protection and availability of data and funding.

In *pharmaco-epidemiological studies* the traffic accident involvement of drivers using psychoactive medication is compared with a group of drivers not using the medication, to investigate the risks associated with medication use. Valuable information is gathered through databases such as prescription records, dispensing records, police reports, health insurance records, hospital databases or by interviewing people. Some limitations inherent to this kind of studies that could lead to underestimation of accident risks, are: incomplete databases, non-compliance or irregular medication use, in some cases the impossibility to differentiate between risks associated with medication use and risks associated with the underlying disorder, privacy issues,… [3]

Roadside surveys investigate the prevalence of psychoactive substances among the general driving population. Drivers are randomly stopped and tested for the presence of alcohol, drugs and/or medicines. The results of these studies become more representative for the general driving population as the number of included drivers increases, and therefore reduces the selection bias. Another way to make results more representative is by weighing according to traffic flow. [8] Disadvantages of this study design are the high cost because a large number of drivers need to be screened, the high risk of (selection) bias and confounding and the fact that in some countries it is legally not possible to screen drivers without suspicion.

Epidemiological studies may also consider only a *subset of drivers*, for example ‘injured drivers’ where biological samples are collected from drivers admitted to hospital and analysed to assess the involvement of psychoactive substances in accidents. A possible drawback in this kind of study is the fact that certain medicines (for instance opioids) may have been administered prior to the sampling (for instance at the accident site or in emergency room).
Besides analysing biological samples, the prevalence of drugs in various populations can also be assessed by collecting self-reported data. They are conducted over the telephone or in face-to-face interviews. Several limitations should be taken into consideration when interpreting the gathered data: under-reporting of actual use, subjects not willing to reveal all information, misunderstanding of the questions or forgetting events. [9] While the prevalence of drug use might be underestimated if solely based on self-report, such questionnaire data can be useful to gather extra information such as route of administration, dose, time of first use, frequency of use, demographics and other data important for traffic statistics.

Accident risks associated with the use of psychoactive substances can be estimated by comparing the prevalence of psychoactive substances among the general driving population (controls) with their prevalence among drivers who were injured, killed or involved in a traffic accident (cases). When using questionnaire data to calculate accident risks a possible underestimation of the prevalence can occur while collecting biological samples might introduce a high percentage of refusals. As most of the studied substances are illicit, control subjects who are users might be more likely to refuse to give a sample than non-users, resulting in a bias by showing a stronger positive association between the drug and the accident risk than is the case in reality.
A case-control analysis does not necessarily take into account possible tolerance and dose-relationship when estimating the crash risk of a certain medicine. Although this could be seen as a disadvantage of the study design, it may also be a strength since it reflects the real-life situation, in contrast to experimental studies. Another possible pitfall is the fact that the study's statistical power might be poor to detect significant proportional differences. [10]

The results from the case-control studies conducted as part of the European research-project DRUID (Driving Under the Influence of Drugs, alcohol and medicines) showed large variations in the odds ratios for driving under the influence of psychoactive substances. Since it is hard to believe that drivers in different countries show such large differences for the relative risk of serious injury, it is unlikely that these deviations reflect actual differences in relative risk in the different countries. The observed differences between the odds ratios can be partially explained by random and systematic errors. [11]

A *random error* can be defined as variability in sampling. Random errors are statistical fluctuations (in either direction) in the measured data due to the precision limitations of the measurement device. Random errors usually result from the experimenter's inability to take the same measurement in exactly the same way to get exact the same number. Random errors can be evaluated through statistical analysis and can be reduced by averaging over a large number of observations.
A *systematic error* is also called bias. Bias is the difference between an observed value and a true value. Bias is, unlike random error, not affected by sample size, because all of the data are shifted in the same direction (either too high or too low). Systematic errors can be divided into three types: selection bias, information bias and confounding.
Selection bias is the bias that results from an unrepresentative sample, examples are undercoverage (some members of a population are inadequately represented) and nonresponse bias (when individuals are unwilling or unable to participate). In case of epidemiological studies on drugs and driving this can result in an underestimated prevalence of psychoactive substances in general traffic and overestimated odds ratio. [11]
Information bias results from systematic differences in the way data on exposure or outcome are obtained from the various study groups. [11] This may mean that individuals are assigned to the wrong outcome category (due to a measurement error or selective misunderstanding of parts of a questionnaire) leading to an incorrect estimate of the association between exposure and outcome.

Confounding occurs when an observed association is in fact distorted because the exposure is also correlated with another risk factor independently of the exposure under investigation. An unequal distribution of the additional risk factor between the study groups will result in confounding. Gender and age are seen as most commonly found confounding factors in case-control studies. [11] In addition, characteristics of drunk/drugged drivers can be different in different countries. For instance Gjerde et al. [12] have suggested that risk taking behaviour might be more pronounced among drunk drivers in countries where driving after drinking is not socially accepted.

Studies are not always easy to compare for example if the data are from different populations, if different types of samples or detection techniques are used or if samples are tested for different psychoactive substances. [3] For example, even though uniform guidelines were present, this was not sufficient to exclude differences in the design and protocol of the six national DRUID case-control studies. Deviations from the guidelines were caused by practical, financial and legal limitations, resulting in random and systematic errors on the odds ratios for driving under the influence of psychoactive substances. [11]

1.2. European epidemiological studies

In Europe there are several individual studies such as in the Nordic countries, [13–17] but there are only a few international surveys. [8,18]

1.2.1. Immortal

IMMORTAL (Impaired Motorists, Methods of Roadside Testing and Assessment for Licensing) was a research program concerning the accident risk associated with different forms of driver impairment and the identification of 'tolerance levels' applied to licensing assessment and roadside impairment testing (including drug screening).
The IMMORTAL-study included case-control studies in The Netherlands, Norway and United Kingdom with a joint research design across all three countries. The study's intention was to examine whether drivers using one or more of a number of defined drug groups, including alcohol, had a higher accident risk than drivers not using these drugs, and to quantify this risk. [8] The main study findings are summarised in Table 1.1.

In the Netherlands and Norway cannabis was the most frequently found illegal substance in the tested drivers, in the United Kingdom amphetamine-type stimulants such as amphetamine and MDMA (3,4-methylenedioxymethamphetamine) were most frequently detected. [8]

Table 1.1. Results of IMMORTAL study: prevalence of psychoactive substances in traffic (%).

Drug alone or in combination	The Netherlands	Norway	United Kingdom
Number of tested drivers	3799	409	1312
Blood alcohol concentration ≥0.2 g/l	2.1	0.0	n.r.
Amphetamine	0.03	0.0	0.66
Benzodiazepines	2.1	0.2	n.r.
Cannabis	4.5	0.5	3.26
Cocaine	0.7	0.0	1.34
MDMA	0.6	0.0	4.61
Opiates (excluding codeine)	0.06	0.2	0.08
Codeine	0.6	n.r.	1.61
Tricyclic antidepressants	0.3	n.r.	n.r.
Methadone	0.04	n.r.	n.r.
Positive for one or more drugs	9.9	1.0	10.8

n.r.: not registered
In Norway, all stopped drivers had to take a breath test for alcohol, but no driver stopped was positive for alcohol above the Norwegian legal limit, BAC 0.2 g/l. In the UK study, blood alcohol concentrations were not registered.

Because of the very limited number of cases in the UK, the injury risk of drug use was only calculated in the Dutch and Norwegian studies. In the Netherlands approximately 35% of serious injuries among drivers (n=184) were positive for alcohol and/or illegal drugs: 12.7% had only BAC (blood alcohol concentration) ≥ 1.3 g/L, 8.3% were positive for drug/alcohol combination at BAC ≥ 0.8 g/L and drug/drug combinations accounted for 7.2%. The corresponding odds ratios were 87, 179 and 24 respectively.
In Norway, of a total of 87 killed or injured drivers, 28 were positive for one or more of following drugs: alcohol, amphetamine, benzodiazepine, cannabis, MDMA and opiates. No case driver was positive for cocaine. The calculated relative risk and odds ratios of drivers, who had used one or more substances are respectively 32.1 and 48.2. [8] Since the number of cases and controls in the Norwegian study was very small, wide confidence intervals were obtained.

1.2.2. DRUID

The European Commission had an objective to halve the number of road deaths between 2001 and 2010, with a target of 27,000. Partly for this reason the project DRUID (DRiving Under Influence of Drugs, alcohol and medicines) was launched in 2006.

Nineteen European countries were involved in the project that was structured in 7 Work Packages (WPs), each having their own objectives and methodology, but connected to each other as part of a dynamic structure (see Figure 1.1). [18]

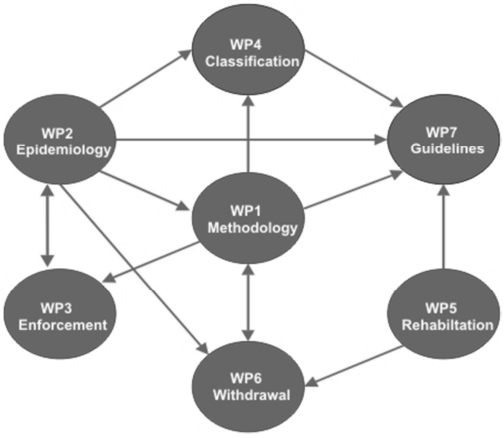

Figure 1.1. Dynamic structure of the work packages in the project DRUID

The target of halving the road deaths was not met; by the end of 2010 a 43% reduction was reached. The Commission proposed to maintain the objective of halving the number of road deaths in the European Union (EU) by 2020, the aim is a maximum of 15,500 traffic fatalities. In 2014 the number of EU fatalities had dropped to 25 700. [1]

This thesis was partly performed with data of the DRUID project, financed by the European Commission. The most relevant work packages (WP2 and WP3) and their outcomes are mentioned in the next paragraphs.

1.2.2.1. WP2: Epidemiology

Roadside survey

In work package 2 of DRUID, a stratified multi-stage sampling design was used to select drivers of passenger cars and vans in a roadside survey, to obtain a number of respondents representative for age, gender and traffic volume over different time periods. One or more regions per country were chosen (representative for the whole country regarding traffic distribution and substance use). Smaller research areas with survey locations were selected to randomly stop drivers and to ask them to participate in the study. Days of the week and times of the day were divided into eight time periods, to be covered by each research area. [19]
In practice it was not possible to distribute the study sample equally with traffic volumes for each of the eight time periods. To correct for this, weight factors were applied by dividing the distribution of traffic by the distribution of the participants, and this for each time period. [19]

The weighted number of positives for a substance was divided by the weighted total of samples to calculate the weighted prevalence for the substance. Ninety-five percent confidence intervals were calculated with the Wilson formula. [19]

For calculating estimates of European mean prevalence, another weight factor had to be used. This factor is ideally based on traffic volume figures for each country. Since these data were not available for all 13 participating countries, population was used as an alternative exposure measure. This weight factor was calculated in several steps. First the number of inhabitants in a participating country was divided by the total number of inhabitants in all participating countries in its European region (North, East, West and South Europe). Secondly a weighted mean per region was calculated based on the weight of each country. Finally a EU weighted mean was calculated by using the weight factor obtained by dividing the number of inhabitants per region by the total population of the EU. [19]

Table 1.2 gives an overview of the prevalence of psychoactive substance groups in the DRUID roadside survey. [19,20]

Alcohol (≥0.1g/L, DRUID cut-off) was the most frequently detected psychoactive substance in the driving population (estimated EU mean 3.48%). It was mainly detected among older male drivers. [19]

The prevalence of illicit drugs (estimated EU mean 1.90%) was lower than the alcohol prevalence. The estimated EU mean prevalence for THC (Δ9-tetrahydrocannabinol) alone was 1.32%, cocaine alone was the second most found with a mean prevalence of 0.42%. The estimated mean prevalence for alcohol (≥0.1g/L) – drug combinations and drug-drug combinations was 0.37 respectively 0.39. [19]

In Belgium almost 7% of drivers tested positive for alcohol (alone or in combination) (≥0.1 g/L), of which 35% had a BAC above 0.5 g/L (the legal limit in Belgium). Benzodiazepines and medicinal opioids were found in 2.3 and 1.0% respectively. THC, cocaine and Z-drugs were found in less than 1% of the cases. [20,21]

Table 1.2. Weighted European mean and Belgian prevalence of substance groups within DRUID Roadside survey.

Substance group	Weighted European mean prevalence (%)	Belgian prevalence (%)	
		Alone	In combination
Negative	92.57	89.35	n.a.
Amphetamines	0.08	/	/
Cocaine	0.42	0.20	0.23
THC	1.32	0.35	0.14
Illicit opiates	0.07	0.09	0.07
Benzodiazepines	0.90	2.01	0.27
Z-drugs	0.12	0.22	0.07
Medicinal opioids	0.35	0.75	0.23
Alcohol	3.48	6.42	0.31
Alcohol-drug combinations	0.37	0.31	n.a.
Drug-drug combinations	0.39	0.30	n.a.

Study in seriously injured and killed drivers

Studies in seriously injured drivers of personal cars and vans were performed in six countries and on killed drivers in four countries. The percentage of subjects testing positive for at least one psychoactive substance ranged between 28 and 53% in injured drivers and 31 to 48% in killed drivers.

As suggested from previous studies, [8,22] alcohol was the most common toxicological finding, both in the seriously injured and in killed drivers (range 17.7 - 42.5% and 19.0 - 44.9% respectively). Other common findings in the injured drivers were THC (range 0.5-7.6%) and benzodiazepines (range 0-10.2%). In the killed drivers study, these were benzodiazepines (range 1.8-13.3%), followed by amphetamine (range 0-7.4%) and THC (range 0-6.1%) (see Table 1.3). [4,5]

Table 1.3. Range of prevalence of substance groups within DRUID study in seriously injured and killed drivers.

Substance group	Injured drivers (range, %)	Killed drivers (range, %)
Alcohol (≥ 0.1 g/L)	17.7 – 42.5	19.0 – 44.9
Amphetamines	0.1 – 4.2	0.0 – 7.4
Cocaine and/or benzoylecgonine	0.0 – 5.4	0.0 – 1.4
THC	0.5 – 7.6	0.0 – 6.1
Illicit opiates	0.0 – 2.1	n.a.
Benzodiazepines	0.0 – 10.2	1.8 – 13.3
Z-drugs	0.0 – 3.8	0.0 – 4.4
Medicinal opioids	0.5 – 7.8	1.7 – 4.1

In Belgium, drivers of other types of vehicles (motorcycle, moped, bicycle, bus and truck) were also included in the study. Within this group (n= 1078), 37 percent of the seriously injured drivers were found positive for one or more (il)licit substances. The highest prevalence was found for alcohol only (20.3%), of which 12% had a BAC between 0.1 g/L - 0.5 g/L and 63.6% had a BAC at or above 1.3 g/L. Five and a half percent of the sampled subjects were positive for medicinal drugs only, mostly benzodiazepines and medicinal opioids. Almost 5% of patients had used only an illicit drug, with the highest prevalence for THC (2.5%). The most commonly observed combinations were benzodiazepines with alcohol (1.9%) and THC with alcohol (1.5%) (see Table 1.4) . [23,24]

Table 1.4. Prevalence of substances groups (alone and in combination) within the Belgian section of the DRUID seriously injured drivers study.

Substance group	Belgian prevalence (%)	
	Alone	In combination
Negative	63.0	n.a.
Amphetamines	0.5	0.9
Cocaine and/or benzoylecgonine	0.4	1.4
THC	2.5	2.8
Illicit opiates	0	0
Benzodiazepines	2.5	2.9
Z-drugs	0.4	0.7
Medicinal opioids	2.3	1.0
Alcohol	20.3	5.9
Alcohol-drug combinations	5.9	n.a.
Drug-drug combinations	1.2	n.a.

Risk estimation

Data of the roadside surveys were compared to data of the injured and killed drivers to calculate odds ratios.

The highest risk of getting seriously injured or killed was associated with driving with high alcohol concentrations (above 1.2 g/L) and alcohol combined with other psychoactive substances. These two groups indicated extremely high risks of about 20-200 times that of sober drivers. Other high-risk groups were drivers with medium blood alcohol concentrations (between 0.8 g/L and 1.2 g/L), multiple drug use and amphetamines. The risks indicated for these groups were about 5-30 times that of sober drivers. Medium increased risk was found for alcohol concentrations between 0.5 and 0.8 g/L, for cocaine, benzoylecgonine, illicit opiates and medicinal opioids. Risk for this group was estimated to about 2-10 times that of sober drivers. The risk associated with cannabis seemed to be similar to the risk when driving with a low alcohol concentration (between 0.1 g/L and 0.5 g/L), which was slightly increased of about 1-3 times that of sober drivers. [25] Table 1.5 shows a summary of the calculated risk of several psychoactive substance groups. [26]

According to the Belgian data, an elevated risk when driving with alcohol concentrations above 0.8 g/L was found. The risk increased drastically with alcohol concentrations above 1.2 g/L. The odds ratios for crash involvement after the use of amphetamine, cannabis, medicinal drugs, alcohol-drug and multiple drug combinations were higher than one. [27]

Table 1.5. Relative risk level of getting seriously injured or killed for various substance groups. [25]

Risk level	Risk	Substance group
Slightly increased risk	1-3	0.1 g/L ≤ BAC < 0.5 g/L Cannabis
Medium increased risk	2-10	0.5 g/L ≤ BAC < 0.8 g/L Benzoylecgonine Cocaine Illicit opiates Benzodiazepines and Z-drugs Medicinal opioids
Highly increased risk	5-30	0.8 g/L ≤ BAC < 1.2 g/L Amphetamines Multiple psychoactive substances
Extremely increased risk	20-200	BAC ≥ 1.2 g/L Alcohol in combination with other psychoactive substances

1.2.2.2. WP3: Enforcement

Driving under influence can be detected by means of a field sobriety test or a drug test. Field sobriety test have shown to lack sensitivity for detecting impairment caused by drug use. [28–30]

The performance of eight on-site oral fluid drug-screening devices was studied in Belgium, Finland and the Netherlands. The main objective was to evaluate the analytical reliability of the devices for testing drivers suspected of driving under the influence of drugs (DUID). The devices were evaluated for the detection of amphetamine(s), cannabis, cocaine, opiates and – if incorporated - benzodiazepines. The results indicated that the opiates tests appeared to perform relatively well when DRUID cut-offs were used. The cannabis, cocaine and amphetamine tests of the devices still lacked sensitivity. None of the devices had more than 80% for sensitivity, specificity and accuracy for all separate tests they comprise. [28,31]

The use of the low DRUID cut-offs in this work package did not give optimal results. Better results were obtained when using the higher Belgian screening cut-offs. [32,33] Based on the screening cut-offs set in this law 4 oral fluid screening devices (Dräger DrugTest 5000, Cozart DDS, Mavand Rapid STAT, and Innovacon OrAlert) were analytically evaluated on 408 volunteers in drug treatment centers. All tests showed good specificity. Dräger DrugTest had the highest sensitivity, although it was still low for some analytes. [33]

More recently Desrosiers et al. [34] conducted an evaluation of an oral fluid on-site test for the detection of cannabinoids. The device had a high sensitivity and the selection of oral fluid confirmation analytes and cut-offs provided appropriate windows of detection to be used in different drug testing programs such as DUID and workplace drug testing. Wille et al. [35] studied another on-site test to detect recent THC use in chronic cannabis smokers. In 90% of the cases the test was positive just after smoking, but sensitivity rapidly decreased to 50% within 1.5 h.

These studies suggest that since 2012 more reliable on-site detection of cannabinoids in oral fluid is available. [36]

1.3. Meta-analyses on DUID

In meta-analyses the results of several studies are combined using statistical methods. They are based on a systematic review, whose quality influences the validity of the meta-analysis. [3]

A meta-analysis on 66 studies was performed by Elvik[37] to investigate if the use of drugs while driving is associated with an increase in risk of accident involvement. Summary risk estimates were described for eleven drugs, all indicating an increase in odds ratio of accident risk. A majority of estimates indicated that the increase in risk is less than a doubling (less than 100%). Studies that scored high on the index of study quality reported lower estimates of risk than studies with lower quality index. The researcher concluded that in future research a better control of confounding factors is needed.

Asbridge et al. [38] conducted a systematic review and meta-analysis on nine studies concerning acute cannabis consumption and motor vehicle collision risk. They concluded that consumption is associated with an increased risk of a motor vehicle crash, which was most evident for high quality studies, case-control studies, and studies of fatal collisions. The impact of acute cannabis consumption on the risk of minor crashes remained unclear.

A meta-analysis of Li et al. [39] also suggested that marijuana use is associated with a significantly increased risk of being involved in motor vehicle crashes in a dose-response way. Analysis resulted in a summary odds ratio of 2.66. [39]

The effects of cannabis on driving skills was also studied by Hartman and Huestis[40] by means of a review of epidemiological and experimental data. The risk of involvement in a motor vehicle accident increases 2-fold after cannabis use. Cannabis-induced impairment was seen with increasing task complexity. Data suggest that blood THC concentrations of 2-5 ng/mL are significantly associated with driving impairment. [40]

Many studies suggest a dose-related risk for cannabis, but a case-control study in Belgium[27] and a responsibility study in France[41] showed that the odds ratio for intermediate THC levels appeared to be higher than for higher levels of THC.

1.4. Limitations of risk estimations and meta analyses

For certain psychoactive substances different odds ratios are observed in different studies. Due to low numbers of positive cases and controls, large confidence intervals are observed and therefore the differences in odds ratios are not statistically significant. [11] The study subjects of different studies can be different, several sociodemographic factors, such as age and gender, can have an influence on the number of positives. For instance a study in France found a higher number of positives for cannabis compared to other similar studies. This was possibly due to the fact that they included only drivers under the age of 30. [42] There can be a difference in the type of psychoactive substances that are targeted, for instance different components out of the group of benzodiazepines, opioids or amphetamines. Some studies measure THC while others use the metabolite THCCOOH to evaluate cannabis use. The latter example illustrates how the use of different biological samples can influence

the results due to the variation in detection time. After cannabis consumption, THCCOOH is detected longer in urine than THC in blood. A last factor that makes it difficult to compare several epidemiological studies is the use of different analytical methods, with different limits of detection (LOD) and quantification (LOQ) and the use of different cut-offs to evaluate positivity for a psychoactive substance (e.g. 0.1 g/L within DRUID and 0.5 g/L within the Belgian law for ethanol). [3]

For meta-analyses, the validity depends on the quality of the systematic review on which it is based. All relevant studies should be covered, heterogeneity should be considered and the main data should be tested for robustness meaning the methodological quality, the design and the performance of each study should be looked into. [3]

2. Quantitative bioanalysis

Epidemiological studies on alcohol and drugs are often performed by collecting self-reported data. A major difficulty with this collection is under-reporting of actual use. [43–46] While under-reporting may be an issue when using questionnaires, high refusal rates may be a problem when collecting samples for bioanalysis. [19]

Bioanalysis is a sub-discipline of analytical chemistry covering the measurement of (amongst others) drugs and their metabolites in biological systems. Reliable data obtained from selective, sensitive and reproducible analysis of a drug and its metabolites in biological samples is a fundamental and crucial part of successful epidemiological studies on drug prevalence such as roadside surveys. Analysis of drug components can be carried out on different biological specimens in a qualitative or (semi)-quantitative way. Qualitative methods only detect the target molecules, meaning only an outcome of present/positive or not-present/negative can be reported. Reporting an exact concentration can only be done based with quantitative methods, using a calibration curve and often using internal or external standardisation. Semi-quantitative results indicate a degree of positivity, without reporting an exact concentration.

Basically, qualitative analysis gives an indication of the identity of the chemical species in the sample, and quantitative analysis determines the quantity of certain components in the sample. [47]

2.1. Biological matrices

Bioanalysis is performed in biological fluids. Blood, oral fluid, urine and hair are the most commonly used biological matrices for drug testing in epidemiological research on drugs of abuse.

The type of specimen and the cut-off concentration must be taken into consideration when planning an epidemiological study on drug use. If recent intake (last intake within 6 to 48 hours ago) is being studied, blood or oral fluid should be collected. If drug use during the last few days is being studied, taking urine samples is better suited for this purpose. If use during the last few months is being studied, hair samples offer a large detection window. The invasiveness and hence the refusal rates are lowest for the collection of oral fluid and highest for blood and hair samples. The costs are lowest for urine and highest for hair. [48]

2.1.1. Blood

Blood and cerebrospinal fluid are the only biological matrices that reflect the drug concentration in the brain, and so the greatest advantage of blood as matrix is the better correlation of the concentration with effect in comparison to other biological fluids. For obvious ethical reasons one cannot take cerebrospinal fluid samples from volunteers in epidemiological studies. The difficulty with blood samples is the invasive collection process that requires qualified personnel and suitable equipment and facilities. In addition high refusal rates have been observed when asking for a blood sample in for example roadside surveys. [19]

No rapid immunoassays that can be used as on-site screening are available for blood and the detected concentrations of substances are low (ng/mL). The detection window is rather short. For instance the length of time cocaine is detectable in blood after single use of 20 mg is 4 to 6 hours and THC is detectable for approximately 5 hours after smoking a joint with a dose between 5 and 30 mg, [49] but in some cases THC was still detectable in the blood of chronic cannabis users up to one month after abstinence. [50] Blood samples are not suitable to study drug use in a wider time-frame. The detection window of psychoactive substances also depends on the analytical sensitivity and the cut-off of the method used.
Since pharmacokinetics for drugs in blood are well studied, correlations between drug concentrations and levels of impairment can be made. Drug concentrations of medicinal drugs might give an indication whether the dose was therapeutic or supra-therapeutic. [48]

Whole blood, plasma, serum and ultra-filtrate of serum are commonly used specimens for drug concentration measurements. Unbound drug molecules in blood distribute themselves among red blood cells, binding proteins (albumin, alpha-1-acid glycoprotein and lipoproteins) and plasma water based on how avidly they partition into red blood cells, the concentration of binding proteins in blood and the affinities of the binding proteins for the drug. For some drugs the concentration in plasma is different from the concentration in whole blood. For instance if the drug concentrates in red blood cells, whole blood concentrations are higher than the corresponding serum or plasma concentrations; when a substance is plasma protein-bound, the concentration in plasma or serum will be higher than in whole blood. [51] Table 1.6 shows blood/plasma ratios for some common psychoactive substances. [52–55]

Table 1.6. Blood/plasma ratio for some (il)licit substances. **[52–55]**

Substance	Blood/plasma ratio
6-monoacetylmorphine	0.57
alprazolam	0.8
amitriptyline	0.83
amphetamine	0.65
cocaine	1.0
codeine	0.87
diazepam	0.55
flunitrazepam	1.3
methadone	0.75
morphine	1.02
nordiazepam	0.59
oxazepam	0.9
THC	0.55
trazodone	0.64
zopiclone	1

2.1.2. Urine

Urine is the most commonly used biological matrix for testing drugs of abuse. Its collection is easier than blood, it is easier to analyse, and a large volume of sample can be taken in which high concentrations of substances are detectable. Other benefits are the fact that a large number of rapid on-site tests is available and that drugs are detectable for a longer time in urine than in blood. [49] The latter can also be a disadvantage since a urine sample of a regular cannabis user can be positive after several weeks or even a few months after termination of use. [56,57]

Although urine is easily sampled, there are privacy issues because medicinal or scientific personnel have to observe the collection to reduce the possibility of adulteration. But refusal rates for given a urine sample may be lower than for blood.
Results do not indicate the dose used and cannot be used to predict time of intake or to assess impairment, since presence of the substance in urine does not imply the presence in blood. [48,58]

2.1.3. Oral fluid

Oral fluid is an easily available medium, collectable without intrusion of privacy. Sampling is easy but in drug users it can be more difficult because of dry mouth and due to the decreased flow rate and a higher viscosity after use of for instance amphetamines or cannabis.
Another advantage of oral fluid is the shorter detection window that correlates with the (impairing) effect and drug findings in oral fluid reflect recent use. Therefore oral fluid is a better choice than urine or hair if studying drug use that may have a pharmacological effect at time of sampling, although some studies on cannabinoids in oral fluid suggest that THC and/or THCCOOH (11-nor-9-carboxy-Δ^9-tetrahydrocannabinol) are still detectable for some days after abstinence in chronic users, even up to 29 days. [59–61]
Oral fluid is difficult to adulterate and on-site screening devices using immunological testing are available for the major drugs. [62] Sensitivity can sometimes be low, [31] but more reliable on-site tests have become available. [34–36]
A positive result in oral fluid reflects presence in blood, indicating drug use within 1-2 days, varying per drug. [49] Since wide variations of oral fluid to blood ratios are observed, no reliable estimation of drug concentrations in blood based on oral fluid concentrations can be made for individuals. [63–66] However, for epidemiological studies, when aggregated concentration data are available, acceptable estimates on drug use for the study population can be obtained, despite the fact that findings for single individuals within the population may be incorrect. [67–69]
For many substances the concentration in oral fluid is much higher than in blood (for instance due to ion trapping of alkaline substances in oral fluid), for other substances the opposite is observed. [62,70]
In the absence of conversion factors, in order to obtain similar prevalence results when using drug concentrations in blood and oral fluid, equivalent cut-offs were calculated within DRUID and used for the final calculations of prevalence.
Table 1.7 gives an overview of the original and equivalent DRUID cut-offs in blood and oral fluid.

Table 1.7. DRUID original and equivalent cut-offs

Substance	Blood cut-off		Oral fluid cut-off	
	Original	Equivalent	Original	Equivalent
6-monoacetylmorphine	10	10	5	**16**
alprazolam	10	10	1	**3.5**
amphetamine	20	20	25	**360**
benzoylecgonine	50	50	10	**95**
clonazepam	10	10	1	**1.7**
cocaine	10	10	10	**170**
codeine	10	10	20	**94**
diazepam*	20	**140**	5	5
flunitrazepam*	2	**5.3**	1	1
lorazepam	10	10	1	**1.1**
MDA	20	20	25	**220**
MDEA	20	20	25	**270**
MDMA	20	20	25	**270**
methadone	10	10	20	**22**
methamphetamine	20	20	25	**410**
morphine	10	10	20	**95**
nordiazepam	20	20	1	**1.1**
oxazepam	50	50	5	**13**
THC	1	1	1	**27**
zolpidem*	20	**37**	10	10
zopiclone	10	10	10	**25**
tramadol	50	50	50	**480**
7-aminoclonazepam	10	10	1	**3.1**
7-aminoflunitrazepam*	2	**8.5**	1	1

* the calculated equivalent cut-off in oral fluid was lower than the original cut-off, therefore the cut-off in blood has been raised.

An issue that could arise when using oral fluid is the lower drug concentrations due to dilution and increasing salivary pH. The ion trapping of basic analytes is highly influenced by salivary pH as a result of changes in the bicarbonate concentration in oral fluid; with higher pH less ion trapping occurs. [71] Other matters concerning oral fluid testing are addressed in Chapter 7, trends in alternative matrices.

2.1.4. Other matrices

Other matrices such as hair or sweat are not frequently used but have also their advantages and disadvantages that are worth mentioning. In hair more of the original molecule and fewer metabolites are found. Also a longer detection time and possible multi-section analysis are advantages of hair analysis. Because hair grows approximately 1 cm/month and the first mm are below the skin surface, it is recommended to collect the hair sample 4-6 weeks after drug intake, especially when a single administration is being investigated. This makes this matrix less suitable for epidemiological research on DUI (Driving Under Influence), because information on recent use is required. There are also problems with external contamination and the influence of cosmetic products. However, it has potential as biological matrix in driver's license regranting procedures. [72–75]

Sweat has the advantage that it reflects recent intake and that sampling can be easily done in a non-invasive way by sweeping the skin, but no standardization on sampling is available. Disadvantages are that there is a possibility of external contamination, low target concentrations are found, no rapid immunoassays are available and no cut-offs have been established. [48]

2.2. On-site testing

Rapid on-site tests are used in combatting driving under influence of drugs. Screening is performed on the spot, and a sample is taken for confirmation if the screening was positive.

Most on-site tests use immunoassay technology for the detection of drugs. This implies that antibodies are present in the test that will bind with the corresponding antigen in the biological fluid. Positive results with this technique are due to either a structural similarity between components or the presence of a functional group on the drug molecule that is recognized by the antibodies.
Rapid immunoassays are available for urine and oral fluid but have so far not been developed for blood samples. However research has shown that a screening device for oral fluid was found to work quite well with post-mortem blood samples in cases of suspicion of drug-related death or when the deceased had a history of drug abuse. [76]

The last published overview of on-site oral fluid screening devices was written for the DRUID-project. Table 1.8 gives an overview of the target compounds and their cut-off values for seven evaluated on-site tests. **[28,31]** Since then manufacturers have continuously improved their devices. Several versions of a test can be available on the international market, adapted to the legal or operational requirements of the country. For example the manufacturer of DrugWipe has a 5S version for Belgium which meets the criteria of the Belgian law and a 3S version approved in the United Kingdom with other cut-offs for Δ9-THC (10 ng/mL) and cocaine (30 ng/mL). (Securetec, personal communication)

Table 1.8. On-site oral fluid screening devices evaluated during the DRUID-project: detected analytes and cut-off values.

Device	**Target compounds (cut-off value in ng/mL) for detected drug group**					
	Amph	Methamph	MDMA	Cocaine	Opiates	Cannabis
Cozart® DDS 806	Amph (50)	Methamph (50)	/	BZE (30)	Morphine (30)	Δ9-THC (31)
DrugWipe® 5+	D-amph (50)	D-methamph (25)	MDMA (25)	BZE (30)	Codeine (10)	Δ9-THC (30)
Dräger DrugTest® 5000	Amph (50)	Methamph (35)	/	Cocaine (20)	Morphine (20)	Δ9-THC (25)
OraLab6	Amph (50)	Methamph (50)	/	Cocaine* (20)	Morphine (40)	Δ9-THC (50)
OrAlert	Amph (50)	Methamph (50)	MDMA (50)	BZE (20)	Morphine (40)	Δ9-THC (100)
Oratect® III	Amph (25)	Methamph (25)	MDMA (25)	Cocaine (20)	Morphine (10)	Δ9-THC (40)
Rapid STAT®	D-amph (25)	Methamph (25)	MDMA (50)	BZE (12)	Morphine (25)	Δ9-THC (15)

Amph = amphetamine, Methamph =methamphetamine, BZE = Benzoylecgonine, Δ9-THC= tetrahydrocannabinol
*target compound not specified by manufacturer.

2.3. Confirmation analysis

The suspicion that drugs may have been consumed and/or the driver was under influence must be confirmed by reliable qualitative and quantitative toxicological analyses. Immunoassays can detect only a limited number of drugs of abuse. They are usually applied roadside or in the laboratory to distinguish between presumably positive and negative samples. All positive results must be confirmed by a more specific technique such as gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-mass spectrometry (LC-MS). Besides confirming immunoassay results, they are used for more comprehensive screening, library-assisted identification, and to quantify drugs and their metabolites in blood, urine, oral fluid, or hair samples. [77]

In forensic toxicology, the trend is shifting more and more to multi-analyte procedures, that have the advantage of detecting more substances in less sample volume in one analytical procedure, with one single sample preparation method.

For blood, sample preparation must be performed before confirmation analysis, usually by protein precipitation (PP, eliminating proteins), liquid-liquid extraction (LLE, extracting a soluble component from a liquid mixture by contact with a second liquid) or solid phase extraction (SPE, using a solid phase and a liquid phase to isolate an analyte from a solution). The common confirmation methods for whole blood have been gas chromatography with single or tandem mass spectrometric detection, but liquid chromatography with single or tandem mass spectrometric detection is increasingly being used for the identification and quantification of a wide range of compounds in biological samples. [78–80] Easier sample preparation, a broader range of drugs that can be detected in one procedure, no need for derivatisation, and short analysis times are the major advantages which allow for both screening and quantitative multi-drug methods. [81] Particularly for multi-analyte procedures, ultrahigh performance LC (UPLC) provides better separation in a shorter time. For example Smink et al. [82] needed 35 minutes for separation of 33 benzodiazepines in blood using classic LC, whereas Ishida et al. [83] needed only 11 minutes for 43 benzodiazepines using UPLC.

The common analytical methods for urine have been immunoassay screening combined with GC-MS confirmation. [84] The limited number of drugs that can be tested by immunoassay is a disadvantage. Hydrolysis of excreted drug conjugates (e.g. glucuronides) and sample preparation by LLE or SPE is common before GC-MS analysis. For LC-MS analysis, dilution and direct injection of urine samples is possible, and in addition direct measurement of glucuronides can be performed. [85]

Sample preparation of oral fluid is commonly performed by LLE or SPE. Dilution or PP might be performed for expectorated oral fluid, while more extensive sample preparation is necessary for oral fluid collected with commercial sampling kits that contain preservatives, surfactants and in some cases dyes which might interfere with instrumental analysis, causing matrix effects. Confirmatory analysis of oral fluid is most often performed with chromatographic-MS techniques. [62] While multi-component GC-methods have been published, the requirement of different derivatisation agents for

different drug classes requires a complex sample preparation. [86] Most new methods are therefore based on LC-MS. [62]

For unknown analyses, GC-MS and LC-MS/MS require comparison of full-scan spectra against pre-established libraries. Operation in full-scan mode greatly reduces sensitivity resulting in missing some significant drugs because they are present in low concentrations. Monitoring only targeted ions (Selected ion monitoring (SIM) in GC/MS and selected reaction monitoring (SRM) in LC-MS/MS) increases sensitivity but knowledge of the compounds to be measured is required. High resolution (HR)/MS with time-of-flight (TOF) or orbitrap instruments offers mass assignment with an accuracy of 0.001 atomic mass units (amu) compared with 1 amu in conventional MS. Tentative identification is thus based on the exact molecular formula instead of a fragmentation pattern. This makes LC-HR/MS more suitable for identification of unknown compounds without the need for availability of a reference standard or a library spectrum. [87]

Several LC–TOF based screening methods have been presented in forensic toxicology. [88–95] LC–TOF methods have been recently improved by the use of data-independent acquisition (DIA), where the simultaneous acquisition of the fragments can be used as an additional identification parameter, which enhances the specificity. [96–98]

3. Pharmacology of psychoactive substances

Pharmacology consists of two concepts: pharmacokinetics and pharmacodynamics. **Pharmacokinetics** describes how the substance is being processed in the body, it describes the time course of a drug in plasma and can be divided into absorption, distribution, metabolism and excretion of the substance.

Absorption is the movement of a substance into the bloodstream from the site of administration (oral, intravenous, subcutaneous, intramuscular, nasal, …). The route of administration is an important part since it has an influence on the bioavailability of a substance to the body. Bioavailability is the fraction of an administered dose of unchanged substance that reaches the systemic circulation. Intravenous administration results in an immediate 100% bioavailability. The degree of absorption and the bioavailability determine whether a certain exposure dose will induce effects and to what extent. It might also explain why the same dose results into toxic effects when administered by one route but not by another. Absolute bio-availabilities of less than 100% are caused by a combination of incomplete gastrointestinal absorption and first-pass metabolism in the liver whereby the concentration of the substance is greatly reduced before it reaches the systemic circulation. For instance first-pass metabolism inactivates morphine due to formation of inactive metabolites, resulting in a low oral bioavailability (around 20%).[99] For those substances that rely on metabolism to produce a desired effect, peak effects will correlate better with the active metabolite than with the precursor form. [100]

During the *distribution* of the substance in the body, in addition to diffusion, binding to components in the blood or cell components in tissues plays an important role. This generally results in the heterogeneous distribution of the substance in the body. For instance, a lipophilic substance will tend to bind to fat and brain tissue.

Concerning *metabolism*, two types of reactions can be distinguished: type-1 (hydrolysis, oxidation, reduction) and type-2 or conjugation reactions (acetylation, glucuronidation, sulfation). The products of type-1 reactions can be inactive or have an active profile that deviates from the activity of the original substance. Products of type-2 reactions are mostly pharmacologically inactive, although there are exceptions, for example the glucuronide form of morphine and midazolam are active.

Elimination of parent drugs and metabolites occurs primarily in urine. [100] Urinary excretion can be influenced by the urinary pH. For instance, amphetamine is excreted almost completely unchanged when urine is kept acidic. Under basic conditions, elimination will be delayed, and metabolism occurs resulting in only a few per cent unchanged excretion of amphetamine. [100] Cocaine, is extensively metabolised, resulting in a low amount of excretion of unchanged substance.

Pharmacodynamics describes the effect of the substance on the body, it relates the plasma concentration to the effect. The ideal pharmacodynamic model states that plasma concentrations are proportional with the effect site (biophase) concentration. This ideal situation, meaning that the same drug concentration will always cause the same effect intensity, can be visualised as in Figure 1 (left diagram). For some psychoactive substances, however, the relationship between concentration and effect is also dependent on the time point after administration. For these cases the concentration-

effect is defined by a hysteresis loop. The same drug concentration in plasma will result in different effect levels at different time points after administration. [101]

Hysteresis loops occur as a consequence of several mechanisms: tolerance, distributional delay, feedback regulation, input and output rate changes, agonistic or antagonistic active metabolites, uptake into active site, slow receptor kinetics, delayed or modified activity, time-dependent protein binding and the use of racemic drugs. Hysteresis loops can be clockwise (the measured effect decreases with time for a given concentration) or counter-clockwise (the effect can increase with time for a given concentration). [101]

A clockwise hysteresis loop can occur when rapid tolerance develops. Tolerance is a time-dependent loss of intrinsic activity that can occur within the time course of a single dose. Examples are cocaine and amphetamines, where concentrations soon after a single dose cause a greater effect than the same concentrations at a later time. [101]

The most common underlying mechanism responsible for a counter-clockwise hysteresis loop is a distribution delay between the systemic substance concentration and the time to reach the effect site. This type of hysteresis has been reported for THC, after smoking and intravenous administration of several doses, indicating the phenomenon is both dose and route independent. [101] The right diagram in Figure 1 shows a counter-clockwise hysteresis for THC; looking at a particular concentration, there is very little effect at one point in time (e.g. t_2), but at a later time (t_5) stronger effects are perceived at the same concentration.

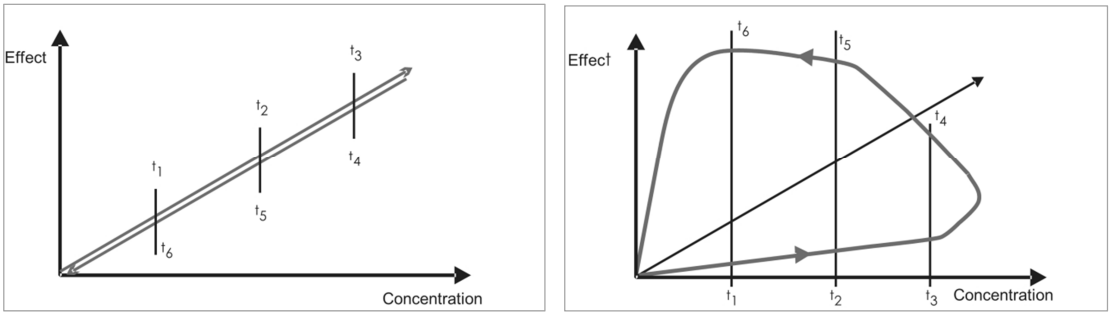

Figure 2. Left: ideal concentration-effect model, right: counter-clockwise hysteresis.

A delay in equilibrium between the plasma and the site of action is also seen with morphine. Since the active metabolite morphine-6-glucuronide (M6G) is highly polar, its passage across the blood brain barrier is difficult. [99] It has been reported that the poor effect of single morphine doses can be explained firstly by the extensive first pass metabolism of morphine to inactive metabolites and secondly by the slow passage of M6G through the blood brain barrier. [99]

3.1. Pharmacological characteristics and effect on driving of some psychoactive substances[53,102,103]

3.1.1 Cannabis

Smoking results in rapid absorption with peak concentrations 10 minutes after smoking. The bioavailability of THC is higher after smoking than following oral administration due to extensive first pass metabolism when ingested. THC is rapidly distributed to tissues. A slow redistribution to plasma of THC sequestered in fat tissues occurs, resulting in a half-life of 3 to 4 days. THC is rapidly and extensively metabolised via cytochrome P450 2C9, 2C11 and 3A isoenzymes in the liver. THC is hydroxylated to 11-hydroxy-Δ9-tetrahydrocannabinol (OH-THC). OH-THC is oxidated to the inactive metabolite THC-COOH. The majority is excreted via faeces and approximately 30% is eliminated in urine predominantly as conjugated glucuronic acids.

THC binds to cannabinoid receptors. Receptor distribution correlates with brain areas involved in physiological, psychomotor and cognitive effects.

Low doses of THC moderately impair cognitive and psychomotor tasks associated with driving. Severe impairment is observed with high doses, chronic use and in combination with alcohol. The more difficult and unpredictable the task, the more likely marijuana will impair performance. [53] The use of cannabis can result in decreased car handling performance, increased reaction times, impaired time and distance estimation, inability to maintain headway lateral travel, subjective sleepiness, motor incoordination and impaired vigilance. [104]

3.1.2. Amphetamines

Amphetamines have good oral and intranasal bioavailability. Peak blood concentrations are seen within 1 to 3 hours after a single dose. After injection, peak concentrations are observed after 15 minutes. The duration of effect of a single dose is 7 to 12 hours, but when urine is alkaline, the half-life may increase due to the basic nature of amphetamines. Amphetamines are metabolised by the liver by a range of enzymes including CYP 2D6 into, amongst others, 4-hydroxyamphetamine, 4-hydroxynorephedrine, benzoic acid and benzyl methyl ketone. Substantial amounts of unchanged drug are excreted in urine, if the urine is acidic.

Amphetamines increase the activity of monoaminergic systems by increasing the release of dopamine, noradrenaline and serotonin from nerve terminals. Noradrenaline is responsible for amphetamine's alerting, anorectic, locomotor and sympathomimetic effects; dopamine stimulates locomotor effects, psychosis and perception disturbances; and serotonin is responsible for delusions and psychosis.

After the use of amphetamines, effects on cognitive functioning are observed, with risk-taking increasing after higher doses, resulting in inappropriate responses to driving issues. In addition, the effects experienced in the withdrawal phase (fatigue, confusion, agitation, lack of concentration) produce impairment of driving ability. [53]

3.1.3. MDMA

The primary route of administration of MDMA is oral ingestion. The onset of action appears within 30 minutes, with peak serum levels after 1 to 3 hours. Elimination half-life is approximately 7 hours, but (as for amphetamines) it can be increased due to alkaline urine. MDMA is metabolised in the liver mainly by CYP 2D6, to an active metabolite methylenedioxyamphetamine (MDA). Additional MDMA metabolites include 3-hydroxy-4-methoxymethamphetamine (HMMA) and 3,4-dihydroxymethamphetamine (HHMA). These polar hydroxylated metabolites are conjugated prior to excretion in urine.

MDMA increases the amount of serotonin, dopamine and noradrenaline released into the synapse.

After the use of MDMA acute changes in cognitive performance and impaired information processing occur. Basic vehicle control is moderately affected but higher levels of risk taking are observed. [53]

3.1.4. Cocaine

Intravenous administration results in 100% bioavailability, snorting in 57% and smoking in 70%. Cocaine is rapidly absorbed after smoking, with peak plasma concentrations occurring within 5 minutes. After snorting absorption is delayed due to the vasoconstrictive action of the drug, with peak concentrations observed after 30 to 60 minutes. Blood levels diminish after as little as one hour. Cocaine is extensively metabolised to the centrally inactive benzoylecgonine, ecgonine and ecgonine methyl ester and to the active norcocaine, a minor metabolite which is neurotoxic. Excretion of cocaine and metabolites occurs through urine by simple filtration.

Cocaine blocks the dopamine transporter, preventing the reuptake of dopamine, leading to increased extracellular dopamine, resulting in chronic stimulation of postsynaptic dopamine receptors (euphoric ‘rush’). A dysphoric ‘crash’ is observed when dopamine levels fall. Cocaine also interferes with the uptake of noradrenaline and serotonin. In addition, it blocks sodium channel activity, explaining its effect as a local anaesthetic.

Cocaine can have the following effect on driving ability: speeding, losing control over the vehicle, high risk behaviour, inattentive driving, and poor impulse control. As the effects wear off, fatigue, depression, sleepiness and inattention can occur. [53]

3.1.5. Heroin (and morphine)

Heroin is smoked, snorted or taken intravenously. After intravenous administration, peak plasma concentrations are obtained within 2 minutes and heroin is no longer detectable after 30 minutes. Heroin is almost immediately metabolised to 6-monoacetylmorphine (6-MAM), which is detectable at 1 minute and peaks at 5 minutes. 6-MAM is rapidly transformed to morphine. Half-lifes for heroin, 6-mam and morphine are approximately 3, 10 and 120 minutes respectively. Both heroin and 6-MAM are more lipid soluble than morphine and enter the brain more readily. Smoking of heroin is half as efficient as the intravenous use. When inhaled, heroin is likely to reach the brain within 20 seconds. Morphine is almost completely metabolised by glucuronidation at the 3- and 6- position. These

glucuronides are rapidly excreted in urine. Morphine can be detected up to 2 days in urine, 6-MAM only for 2 to 8 hours following heroin use, although in some users no 6-MAM is detected.
Heroin has little affinity for opiate receptors and most of its pharmacology resides in its metabolism to active metabolites, namely 6-MAM, morphine and morphine-6-glucuronide. Morphine produces its major effects on the central nervous system through μ-receptors, and also at κ- and δ-receptors. These receptors are involved in pain modulation, analgesia, respiratory depression, miosis, euphoria, decreased gastrointestinal activity, drowsiness, nausea, mental clouding, diuresis, sedation, dysphoria, delusions and hallucinations.
Slow driving, wearing, poor vehicle control, poor coordination, slow response to stimuli, delayed reactions, difficulty following instructions and falling asleep at the wheel, can occur after use of heroin (morphine). [53]

3.1.6. Benzodiazepines

Benzodiazepines are well absorbed orally and reach peak plasma concentrations within 30 minutes to a few hours after ingestion. The metabolism of the different benzodiazepines occurs through either the oxidative microsomal enzyme system or by conjugation with glucuronic acid. Hydroxylated or dealkylated metabolites are often biologically active (e.g. nordazepam, oxazepam, desalkylflurazepam) and increase the duration of the action of the substance. Clonazepam and flunitrazepam are extensively metabolised to 7-amino and 7-acetamido analogues. The elimination of the metabolites occurs primarily through urine with often little unchanged parent drug excreted.
Benzodiazepines bind to the β-subunit of the $GABA_A$ receptor complex, which results in an increase in affinity for GABA. Therapeutic effects of benzodiazepines include sedation, hypnosis, muscle relaxation and anxiolysis.
The most important effects of benzodiazepines on driving performance are somnolence, sedation, and loss of motor coordination, memory impairment, behaviour disinhibition and paradoxical agitation. The highest accident risk is observed in the first weeks of treatment. [105]

3.1.7. Zolpidem (as example of Z-drugs)

Zolpidem is taken orally. First-pass hepatic metabolism results in an oral bioavailability of 67%. Ninety-two percent is bound to proteins in plasma. Peak plasma concentrations are detected at 1.5 to 2.5 hours. Zolpidem is hydroxylated by cytochrome P450 3A4 isoenzymes. The half-life is approximately 2 hours.
Zolpidem is a $GABA_A$ receptor agonist and binds to receptors containing a α_1-subunit.
After a dose of 10 mg, zolpidem causes effects when driving within 5 hours of use. Zaleplon causes impairment within 3 hours of use. Zolpidem and zaleplon are relatively free of residual morning-after effects. Zopiclone causes severe impairment 1 to 5 hours after dosing (7.5 mg), with residual hangover effects up to 11 hours. Examples of possible effects on driving are: weaving, lane travel, slow reflexes, dazed appearance, disorientation, somnolence, double vision, and poor performance on field sobriety tests, poor attention and an inability to stand or walk unassisted. [53]

4. Legislation on DUID

Legislation on DUID can be based on per se laws or on impairment.

When the impairment approach is used, prosecution is based on the fact that the driver shows clear symptoms of impairment, either in behaviour or driving style. It must be proven in each single case that the driving skills of the driver are affected negatively. For this, fixed protocols are mostly used but in most cases they are derived from protocols to detect alcohol impairment and are therefore not optimised for detection of drug effects. [106]

When the approach is based on per se limits, prosecution is based on the fact that a drug is found in the driver's body fluid above a defined cut-off value. Zero tolerance laws are a specific subcategory of per se legislation with a substance concentration of zero, so any detectable amount of legally named psychoactive substances is considered a law violation.
Three categories of thresholds for substances can be defined. Risk thresholds: blood concentrations related to a certain accident risk or impairment driving; lower effect limits: lowest concentration for which an effect on driving could be reported; limits of detection: concentrations based on technical limitations in order to have a valid, reliable result with the lowest percentage of false positives possible.
Countries with zero tolerance legislation use, until now, analytical cut-offs, meaning the concentrations that can be reliably determined by forensic laboratories. In some countries, some consideration was given to the effect of the substances, for instance by not measuring inactive metabolites of cannabis, but only the active THC. [106]

The approach is called a two-tier system when a combination of per se limits and an impairment approach is used. Since no clear solution to link substance consumption with levels of impairment is available, this system seems the most favourable to combat DUID. The combination of a per se legislation and an impairment law, allows to set per se limits to a few substances (e.g. most common drugs) and to use an impairment approach for less frequent cases such as medicinal drugs, since it is not realistic to develop thresholds for all these medicinal substances. For most psychoactive substances there is no clear linear relationship between concentration and effect. Effects are also person-dependent meaning that for instance the same concentration could have a low effect in a tolerant individual and high effect in a drug-naïve person. [106]

Per se laws establish a fixed substance limit and if the driver is detected with a substance concentration reaching or exceeding the limit, he has committed a law violation without the necessity of showing any further signs of impairment.

The United Kingdom (UK) approach is an example of a per se law taking into account analytical and impairment issues: setting a limit at a level that does not catch someone who has consumed a very small amount of the drug in question. A tough approach will therefore not necessarily mean setting limits to zero. It involves setting a limit at the lowest level at which on one hand a valid and reliable

analytical result will be obtained, yet above which issues such as passive consumption or inhalation can be ruled out, a so called 'lowest accidental exposure limit'. [107]

Table 1.9 gives an overview of legal regulations in the European countries based on a survey conducted in 2011 and updated for Norway and the United Kingdom to the situation in 2015. [106,108]

Table 1.9. Legal approaches regarding DUID in European countries, situation in 2011, updated for Norway and United Kingdom (situation 2015) **[106,108,109]**

Country	Legal approach		Country	Legal approach	
	Impairment	Per se limits		Impairment	Per se limits
Austria	X		**Italy**		X
Belgium	X	X	**Latvia**	X	X
Bulgaria		X	**Lithuania**		X
Croatia		X	**Luxembourg**	X	
Cyprus	X	*	**Norway**	X	X
Czech Republic	X	X	**Poland**		X
Denmark	X	X	**Portugal**		X
Estonia		X	**Slovakia**	X	X
Finland	X	X	**Slovenia**		X
France		X	**Spain**	X	X
Germany	X	X	**Sweden**		X
Greece	X		**Switzerland**	X	X
Hungary		X	**The Netherlands**	X	*
Ireland	X		**United Kingdom**	X	X

*Coming soon

4.1. Situation in Belgium

In Belgium a two-tier system is being used. [32,110] Drivers are first observed for external signs of recent drug use applying a fixed protocol, using a checklist. Afterwards if justified by the checklist, an on-site drug test is performed. A biological sample is taken when the screening test is positive and analysed in an accredited lab. Analytical cut-offs are applied, but in se the law is a zero-tolerance law.

In Belgium there is a legal regulation on fitness to drive, based on the EU directive. [111] In case of addiction or abuse of psychoactive substances such as alcohol and drugs, a person is stated not fit to drive. This applies also for people using psychoactive medicines who could have a negative effect on driving ability.

The royal decree on the general regulation when using the public road of 1975 states a driver should be fit to drive and have his/her vehicle under control (article 8.3). [112] An annex of the Royal Decree on the driver licence of 1998 describes the minimum medical norms/standards to which a driver should comply to be assessed as fit to drive. [111]

In 2009 a new law was passed in Belgium concerning the introduction of oral fluid on-site testing for drugs in traffic. [32] Belgium is one of the first countries implementing a DUID-law based on oral fluid for both screening and confirmation. In Belgium, previously a person suspected to be driving under influence of an illegal substance mentioned in the Belgian Law had to undergo urine sampling and confirmation analysis was performed on blood. If the roadside urine test was positive, the driver's license was withheld for 12 hours. [113]

Since October 2010, the new law became effective. [32] First the driver is being assessed by a police officer for signs of recent drug use. For this a standardised checklist is being used. [110] The checklist has been prepared by a working group of law enforcement officers; it is based on international guidelines, which were evaluated in other countries, as well as within DRUID. [28] The list is being checked in its totality and all the signs that are assessed are being marked. At least three signs spread over at least two different categories have to be marked to consider an indication of sign of recent use (see Table 1.10).

If signs of recent drug use are suspected, an on-site oral fluid test is performed.

When the result is at or above the cut-offs set in the law, a second oral fluid sample is taken for the confirmation analysis. If an oral fluid specimen cannot be collected, a blood sample is taken instead for confirmation purposes. Cut-offs for the on-site test and the confirmation analyses on both oral fluid and blood (plasma) are given in Table 1.11, as well as some other international cut-offs.

Table 1.10. Belgian standardised checklist for assessing signs of recent drug use[110]

EYES		STATE OF MIND	
Shiny eyes	□	Euphoria	□
Weepy/teary eyes	□	Tears	□
Blurry eyes	□	Changing (unpredictable) mood	□
Bloodshot eyes	□		
Constricted pupils	□	LANGUAGE	
Dilated pupils	□	Stammering/stuttering	□
Slow light reaction of the pupils	□	Constantly repeating the same words	□
No light reaction of the pupils	□	Flood of words/ verbiage	□
Hypersensitive to light	□		
Drooping eyelids	□	WALK	
		Hopping	□
FACE		To confident/assertive/decided	□
Dry mouth/lips	□	Looking for support, stumbling, wobbling	□
Dried saliva around mouth	□		
Damaged teeth (brownish, becoming black, missing, loose teeth)	□		
		OTHER	
Grinding teeth	□	Visible pulsating veins	□
Presence of the product on the nostrils	□	Trembling limbs (hands, arms, legs)	□
Pale skin colour	□	Disorientation in time and space	□
Repeated snorting	□	Sweat	□
		Twitch	□
BEHAVIOUR		Accelerated reflex	□
Excited/nervous	□	Delayed reflex	□
Verbal/physical aggression	□	Admits using drugs within the past 12 hours	□
Mental confusion	□	Scent of the product (cannabis, chemical)	□
Resignation	□	Possession of drugs or paraphernalia	□
Fatigue	□		

Table 1.11. Cut-offs of substances recorded in the law and literature. SAMHSA & AS4760 are intended for workplace testing with lower cut-offs for a longer detection window.

Substance	Belgian cut-off (ng/mL) for the onsite oral fluid test[32]	French cut-off (ng/mL) for the onsite oral fluid test[114]	Belgian cut-off (ng/mL) for oral fluid confirmation analysis[32]	Belgian cut-off (ng/mL) for plasma analysis[32]	SAMSHA confirmation cut-off for oral fluid[62]	Talloires confirmation cut-off for oral fluid/ blood[115]	AS4760 Australia confirmation cut-off for oral fluid[116,117]	DRUID cut-off oral fluid / blood[19]
THC	25	15	10	1	2	2/1	10	27 / 1
Amphetamine	50	50	25	25	50	20/20	25	360 / 20
MDMA	50	50	25	25	50	20/20	25	270 / 20
Morphine	10	10	5	10	40	20/10	25	95 / 10
6-acetylmorphine	10	10	5	10	4	5/10	10	16 / 10
Cocaine	20	10	10	25	8	10/10	25	170 / 10
Benzoylecgonine	20	10	10	25	8	10/50	25	95 / 50

4.2. Other European countries

In ***Denmark*** all illegal and legal drugs with abuse potential (e.g. opioids) are forbidden in the traffic above a fixed concentration limit in whole blood. Exceptions are made for legal drugs in case one has a prescription and one is judged able to drive in a sure manner (investigated by a medical doctor).
For therapeutic drugs, e.g. morphine, the limits were selected based on the lower therapeutic limits taken from the literature. Concerning illegal drugs with no therapeutic use, the lower limit for pharmacological effect was used if available or a concentration level documented to correspond with intake of usual abuse doses (e.g. drugs as cathine or cathinone). For the most frequently abused drugs that have limits established in other countries, these limits were also taken into account. Table 1.12 shows the substances mentioned in the legislation of 2007, [118] in 2011 this list was extended with amongst others several 'new psychoactive substances'. [119]

Table 1.12. Cut-offs for confirmation (blood) in the Danish DUID-legislation (list of 2007) **[118]**

Drug taken	Active component	Legal limit (mg/kg blood)	Drug taken	Legal limit (mg/kg blood)
List A			**List E**	
Cannabis	THC	**0,001**	Alprazolam	0,005
Diacetylmorphine (heroin)	Morphine	**0,010**	Amphepramone	0,003
Lysergic Acid Diethylamide (LSD)		**0,0005**	Barbital	5,0
Opium	Morphine	**0,010**	Bromazepam	0,050
			Brotizolam	0,002
List B			Chlordiazepoxide	0,20
Amphetamine		**0,020**	Clobazam	0,10
Cocaine		**0,020**	Clonazepam	0,005
Dextromoramide		**0,075**	Diazepam	0,100
Dextropropoxyphen		**0,050**	Estazolam	0,050
Fentanyl		**0,001**	Lorazepam	0,020
Hydrocodone		**0,010**	Lormetazepam	0,005
Hydromorphone		**0,010**	Meprobamate	5,0
Ketobemidon		**0,025**	Midazolam	0,050
MBDB		**0,020**	Nitrazepam	0,020
MDMA		**0,020**	Nordazepam	0,100
Metamphetamine		**0,020**	Oxazepam	0,100
Methadone		**0,050**	Phenobarbital	10
Methylphenidate		**0,010**	Phentermine	0,030
Morphine		**0,010**	Temazepam	0,020
Oxycodone		**0,010**	Triazolam	0,002
Oxymorphone		**0,010**	Zolpidem	0,080
Pethidine		**0,10**	Zopiclon	0,010
PMA		**0,020**		
PMMA		**0,020**		
Tetrahydrocannabinol (THC)		**0,001**		
List D				
Buprenorphine		**0,0005**		
Flunitrazepam		**0,005**		
Pentazocine		**0,010**		
Pentobarbital		**1,0**		

In 2008, ***France*** passed a law with roadside oral fluid screening and confirmation in blood. [114,120] At the moment, an evaluation is ongoing to introduce oral fluid as confirmation matrix. Screening cut-offs and per se limits in blood are given in Table 1.13.

Table 1.13. Cut-offs for screening (oral fluid) and confirmation (blood) in the French DUID-legislation[120]

Substance	Screening cut-off in oral fluid (ng/mL)	Confirmation cut-off in blood (ng/mL)
THC	15	1
(Meth)amphetamine	50	50
MDMA	50	50
Cocaine	10	50
Benzoylecgonine	10	50
Morphine	10	20
6-monoacetylmorphine	10	20

In ***Germany*** the Road Traffic Act there is a zero-tolerance for driving under the influence of the main illegal drugs listed in an annex. Expert recommendations for analytical cut-offs in blood serum were implemented, based on technical limitations and guaranteeing a valid and reliable result. In Table 1.14 the German confirmation thresholds are given.
After stopping a driver there must be a reasonable suspicion before drug or alcohol testing is applied. The German police performs drug recognition tests and urine or oral fluid on-site tests. Different procedures and tests are used in the different states since police work is the responsibility of the Federal Länder. (Martina Albrecht, personal communication)

Table 1.14. Cut-offs for confirmation (serum) in the German DUID-legislation.

Analyte	Cut-off in serum (ng/mL)
D9-Tetrahydrocannabinol	1
Morphine	10
Benzoylecgonine	75
MDMA	25
MDEA	25
Amphetamine	25

Currently discussions are held regarding the cut-off for cannabis. It is specified in the German driving licensing regulation that cannabis consumption has to be separated from driving. Using an analytical cut-off of 1 ng/mL can result in positive serum samples while consumption was actually separated from driving. The general recommendation of the Federal Highway Research Institute (BASt) is to align the regulations for cannabis with those for alcohol: the lowest concentration having a scientifically proven effect on driving performance should be used as a threshold. (Anja Knoche, personal communication).

In ***Italy***, when driving under influence of drugs is suspected, drivers are accompanied to the health department in order to give a biological fluid for analysis. The law states that appropriate biological fluids must be analysed to judge impairment but the types of bio-fluids are not specified.

For the on-site screening, oral fluid tests are allowed, but they have not been implemented by most of police departments.

Urine samples are analysed to get information on what class of substances are putatively present. Substances and their metabolites are quantitatively confirmed in both urine and blood. There are no cut-offs mentioned in the law, the different toxicology departments use their own cut-off, that could be either a LOD (limit of detection) or a LOQ (limit of quantification) or an interpretative cut-off. There is no consensus, but the Italian association of forensic toxicologists has compiled some guidelines for laboratories that are performing analyses with a forensic concern, with suggested cut-off for blood analysis that should be interpreted as minimum required performance limits for hospital laboratories (see Table 1.15). [121] (Donata Favretto, personal communication)

Table 1.15. Minimum required performance limits for hospital laboratories proposed by the Italian association of forensic toxicologists. **[121]**

Substances	**Cut-offs (ng/mL)**				
	Urine screening	Urine confirmation	Blood confirmation	Oral fluid screening	Oral fluid confirmation
Morphine	300	100	10	40	40
Codeine		100	10		40
Monoacetyl-morphine		10	10		4
Cocaine		100	10		8
Benzoylecgonine	300	100		20	8
Amphetamine	500	200	20	50	50
Methamphetamine	500	200	20	50	50
MDMA	500	200	20	50	50
Methadone	300	100	10		
THCCOOH	50	15			
THC			2	4	2
Buprenorphine	5	5			

In ***Norway*** impairment based legislative limits for driving under the influence of non-alcohol drugs are implemented (see Table 1.16). [122] Impairment limits, representing drug concentrations in blood likely to be accompanied by a degree of impairment comparable to a BAC of 0.2 g/L, were implemented for 20 psychotropic drugs, including the most prevalent benzodiazepines, cannabis, GHB (gamma-hydroxybutyric acid), hallucinogens and opioids. Limits for graded sanctions, representing drug concentrations in blood likely to induce impairment comparable to BACs of 0.5 g/L and 1.2 g/L were defined for 13 of the 20 substances. The suggested limits were based for some substances on assessments of impairment after single doses of the drugs in naïve individuals. For other substances the limits were set at a fifth of a concentration accompanying the use of 'standard' recreational and inebriating doses. The proposed limits do not apply to individuals with valid prescriptions for medicinal drugs, where a system with individualized expert evaluations will be maintained. Police officers still use field impairment testing (standardised sobriety tests) to decide whether a driver is impaired by drugs or alcohol. (Hallvard Gjerde, personal communication April 2014), but the revised DUI law opened the possibility of using oral fluid testing as a screening method before taking a blood sample.

The Norwegian mobile police service recently decided to purchase the Dräger Drug Test 5000 instrument for drug screening. (Hallvard Gjerde, personal communication June 2015).

Table 1.16. Cut-offs for confirmation (blood) in the Norwegian DUID-legislation[122]

Substance	Low limits (ng/mL in whole blood)	Impairment limits comparable to a BAC of 0.5 g/L (ng/mL in whole blood)	Impairment limits comparable to a BAC of 1.2 g/L (ng/mL in whole blood)
Benzodiazepines and benzo-like			
Alprazolam	3	6	15
Clonazepam	1.3	3	8
Diazepam	57	143	342
Phenazepam	1.8	5	10
Flunitrazepam	1.6	3	8
Nitrazepam	17	42	98
Oxazepam	172	430	860
Zolpidem	31	77	184
Zopiclone	12	23	58
Cannabis			
THC	1.3	3	9
Central stimulants			
Amphetamine	41	*	*
Cocaine	24	*	*
MDMA	48	*	*
Methamphetamine	45	*	*
GHB			
GHB	10 300	30 900	123 600
Hallucinogens			
Ketamine	55	137	329
LSD	1	*	*
Opioids			
Buprenorphine	0.9	*	*
Methadone	25	*	*
Morphine	9	24	61

* Limits have not been suggested because the correlation between drug concentration and risk of traffic accidents/impairment is variable or insufficiently documented.

Since December 2010 ***Spain*** also modified its law to be able to use oral fluid as screening and confirmation matrix. [123,124]

Drivers of motor vehicles and motorcycles can be screened for drugs, narcotics and psychotropic substances. Firstly some physical tests are performed (appearance of the eyes, behaviour, walk,…) after which an on-site test is conducted by officers of the judicial traffic police. When the oral fluid screening test (which the driver is obliged to undergo) yields a positive result, or when the driver shows signs of having used a psychoactive substance, an oral fluid sample is taken for analysis in a certified laboratory for confirmation purpose. Every driver may request a contra-expertise blood or urine sample. Most cases involve simply an administrative penalty (a fine of 1000 euros and

withdrawal of 6 points on drivers licence). Only in very specific cases, the offence falls under criminal law with a sentence of 3 to 6 months in prison and withdrawal of license as consequences. In Table 1.17 the legal limits in Spain are given.

Table 1.17. Cut-offs for screening (oral fluid) in the Spanish DUID-legislation[123,124]

Substance	Screening cut-off in oral fluid (ng/mL)	Confirmation cut-off in oral fluid or blood (ng/mL)
THC	25	Depending on the LOD of the routine method of the laboratory performing the analysis
Amphetamine	50	
Methamphetamine	35	
Cocaine	20	
Morphine	20	

In 1999 ***Sweden*** implemented zero-concentration limits for controlled drugs in the blood of drivers. The prosecution case is simplified, since it rests primarily on the forensic toxicology report meaning there is no more need to prove that a person's ability to drive safely was impaired. If the drug is taken according to a medical prescription and the person was not considered unfit to drive, the case is exempt from the zero-limit law. [125] The detection of driving under the influence is performed by an eye examination, followed by further examination if there is reasonable suspicion. [126]

In ***The Netherlands*** traditionally legislation is based on impairment and legal limits were set only for ethanol. The new legislation will, besides the introduction of oral fluid testing, adopt impairment thresholds for the single use of most common drugs in blood. In cases of combined use of these substances or in cases of combination with alcohol, the legal limits of these substances are set at zero tolerance. Therefore additional analytical thresholds have been determined as the lowest concentrations for which laboratories could measure reliable and reproducible results. For substances with influence on driving ability for which no thresholds are included in the legislation, impairment stays the reference for penalisation, based on measured concentrations in blood and an expert report in which a toxicologist assesses the effects on driving ability. [127–129]
Table 1.18 gives an overview of the impairment(single use) and analytical (combined use) cut-offs.

Table 1.18. Cut-offs for confirmation (blood or plasma) in the Dutch DUID-legislation. [128,129]

Substance	Cut-offs in blood (b) or plasma (p) (µg/L)	
	Single use	Combined use
Amphetamine	50	25
Methamphetamine	50	25
MDMA	50	25
MDEA	50	25
MDA	50	25
THC	5 (p); 3 (b)	1
Cocaine	50	10
Heroïne	20	10
Morphine	20	10
GHB	10 mg/L	5 mg/L
Ethanol		0.2 g/L

In March 2015 the ***United Kingdom*** moved from impairment legislation to per se enforcement on DUID. [130,131] The idea behind the approach is to set for 8 controlled drugs associated with illegal use, a limit at the lowest level at which a valid and reliable analytical result can be obtained, yet above which issues such as passive consumption or inhalation can be ruled out - a 'lowest accidental exposure limit' and to set expert panel recommended limits for further 8 controlled drugs which have medical uses based on a road safety risk approach. [107,132] These per se limits are given in Table 1.19.

Table 1.19. Cut-offs for confirmation (blood) in the UK DUID-legislation. [131]

Controlled drug	Limit (µg/L blood)
Benzoylecgonine	50
Cocaine	10
Delta-9-Tetrahydrocannabinol	2
Ketamine	20
Lysergic Acid Diethylamide	1
Methylamphetamine	10
Methylenedioxymethamphetamine	10
6-Monoacetylmorphine	5
Morphine	80
Clonazepam	50
Diazepam	550
Flunitrazepam	300
Lorazepam	100
Methadone	500
Oxazepam	300
Temazepam	1000

The police uses an on-site oral fluid test to establish the presence of cocaine and cannabis. [133] Until approval for the screening of other substances is given, police forces will still carry out an impairment test.

Objectives and outline of present dissertation

This thesis is partly performed with data collected within the EU project DRUID, specifically in the Work Packages 2 and 3.

Another part is based on data of DUID cases analysed by the toxicological laboratory of the National Institute for Criminalistics and Criminology.

Several research questions were defined:

What's the effect of the new Belgian DUID law based on oral fluid? Is there a difference in number of confirmed positives when comparing the quantitative results of paired samples of blood and oral fluid? What is the effect on the number of drivers testing positive when using similar cut-offs in blood and oral fluid?

> Chapter 2 describes the comparison of confirmation results in paired samples of blood and oral fluid, to investigate whether more or less drivers would test positive if oral fluid is used as confirmation matrix.

Is there a difference in number of true and false positives between the previous and current legal approach on DUID in Belgium? What is the effect of the change in screening procedure and the lowering of the confirmation cut-offs in blood?

> Chapter 3 compares the number of true and false positives between two legal approaches with different screening methods and confirmation cut-offs, to study if the total number of false positive screenings has dropped since the new legislation is applied, to see if this expected decrease is true for all substance classes and to investigate which change in the legislation has led to the increase of true positives: the change of screening procedure or the lowering of the confirmation cut-offs.

When conducting risk analysis, only prevalence of use is compared between cases and controls, meaning a case or control is categorised as positive (above a set cut-off) or negative for a certain psychoactive substance. What if quantitative analysis is not only used to see if a concentration is under or above a certain cut-off, but to compare the distribution of concentrations between the general driving population and injured drivers? Which conclusions could be drawn: are the concentrations in the latter group, as to be expected, higher? Which conclusions can be drawn regarding medicinal drugs?

> In chapter 4 the concentrations of psychoactive substances between the general driving population and injured drivers are compared, to investigate the expected trend that higher concentrations would be detected in the blood samples of injured drivers.

Is there a good correlation between ‘self-reported use’ of cannabis and toxicological data? In many epidemiological studies the prevalence of drug use is assessed by collecting self-reported data. By additionally performing analyses on biological samples, the validity of these self-reported data can be examined. We will investigate if there is a good match between results of blood and oral fluid analyses on one hand, and on the other hand, self-reporting by means of a questionnaire, in a subpopulation of drivers. Can self-reporting replace analysing biological samples in the domain of DUID?

> Chapter 5 describes the correlation between ‘self-reported’ use of cannabis and the results of bioanalysis in a group of randomly selected drivers. Are there discrepancies between self-report and quantitative analyses in blood or oral fluid and is this related to the time after intake?

Can the time of cannabis use be predicted based on the THC and THCCOOH concentrations found in the samples? And can the results of roadside testing be used to predict the prevalence of drug use in the general population?

> In chapter 6 the time of cannabis use was predicted using the THC and THCCOOH concentrations of positive drivers in a roadside survey and a seriously injured drivers study. The data on cannabis use collected during the roadside study were compared with the data of the Belgian Health Interview Survey collecting information on the use of cannabis in the general population. In addition self-report of psychoactive substance other than cannabis is being discussed

When using the DRUID cut-offs, for several substances a difference in number of positives could be observed when using oral fluid compared to using blood. To neutralize this problem, equivalent cut-offs were set up. Which differences could be seen when using these equivalent cut-offs compared to the original cut-offs for the Belgian data?

> In chapter 7 the calculations using equivalent cut-offs are compared with the original data of chapter 2. This last chapter also summarizes and evaluates the main results. In addition an overview of recommendations is given. Future perspectives on what is ongoing in forensic toxicology (new biological matrices, new psychoactive substances,…) are discussed.

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CHAPTER 2

DUID: Oral Fluid and Blood Confirmation Compared in Belgium

In the Belgian DUID-legislation similar cut-offs for oral fluid and blood samples are used. The effect of using these similar (in order of magnitude) cut-offs in paired samples of oral fluid and blood in a population of general drivers was investigated. The data of the Belgian roadside study, part of the DRUID WP2, were used for this comparison, comprising 2750 respondents for which both blood and oral fluid sample were available.

Using similar cut-offs for confirmation analysis in oral fluid as in blood results in a higher percentage of drivers that confirmed positive.
Due to the low screening cut-offs of the onsite tests, it is necessary to set the confirmation cut-offs at a similar low level (instead of searching for equivalent values), in order to reduce the number of false positive screenings.

Personal input:

- Part of data collection at roadside surveys
- Toxicological analyses
    - 5-10% of oral fluid analyses using UPLC/MSMS for detection of (il)licit drugs
    - 10-15% of alcohol analyses on blood and oral fluid samples using an enzymatic method
    - ELISA and GCMS analyses on blood samples for detection of THC and THCCOOH
- Statistical processing
- Writing the paper

Based on: T. Van der Linden[a,b], S-A. Legrand[a], P. Silverans[c] and A. G. Verstraete[a,d]

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Abstract

The objective of this study was to compare the number of drivers with drug concentrations above the legal cut-offs for driving under the influence of illicit substances in paired samples of blood and oral fluid.

Between January 2008 and September 2009, 2949 randomly selected drivers participated in a roadside survey. Each was asked to provide blood and oral fluid. Samples were analysed for 11 illicit substances or metabolites by ultra-performance liquid chromatography-tandem mass spectrometry and gas chromatography-tandem mass spectrometry.

Out of the 2750 drivers who gave both blood and oral fluid, 28 (1.0%) had drug concentrations above the legal cut-off in blood and 71 (2.6%) were above the legal cut-off in oral fluid. Fifteen (7.5%) of the 199 drivers who gave an oral fluid sample but refused to provide blood tested positive, significantly more than drivers who provided both samples.

Based on oral fluid analysis, 2.6 times more subjects tested positive for drugs compared to blood analysis. Those that refused to give a blood sample were 3 times more likely to test positive for drugs. Even in a survey that guaranteed total anonymity, people fearing a positive test result might have been more likely to refuse to give a blood sample.

Introduction

The risks of driving after consuming alcohol are well known, unlike the dangers associated with using other psychoactive substances.

In 2009, a new law was passed in Belgium concerning the introduction of oral fluid on-site testing for drugs in traffic. [1] Belgium is one of the first countries implementing a DUID-law based on oral fluid for both screening and confirmation. In five Australian states, screening and confirmation are performed in oral fluid. This is different from other countries where confirmation analysis is performed on blood samples. In 2008 France, passed a law with roadside oral fluid screening and confirmation in blood. [2] Norway is planning to implement a law in 2012 based on oral fluid screening and blood confirmation. Since December 2010, Spain also modified its law to be able to use oral fluid as screening and in confirmation analysis, but no legal confirmation cut-offs have yet been established.
Previously in Belgium, a person suspected to be driving under influence of an illegal substance mentioned in the Belgian Law had to undergo urine sampling and confirmation analysis was performed on blood. If the roadside urine test was positive, the driver's license was withheld for 12 hours.
In October 2010, the new law became effective. If the driver is suspected to be under influence (verified by a standardized checklist) of one of the substances, an on-site oral fluid test is performed. When the result is at or above the cut-offs set in the law, a second oral fluid sample is taken for the confirmation analysis. If an oral fluid specimen cannot be collected, a blood sample was taken instead for confirmation purposes. Cut-offs for the on-site test and the confirmation analyses on both oral fluid and blood (plasma) are given in Table 2.1.

Oral fluid offers multiple advantages as an alternative matrix for drug testing: sample collection is easy and non-invasive, which can be observed without the need of special restroom facilities and same-sex collectors. It also reduces the risk of adulteration and infection, and it better reflects recent drug use than urine sampling. The parent drug is prominent in oral fluid and provides some correlation with pharmacodynamic effects such as impaired performance. [3,4]

In oral fluid, ion trapping of basic drugs, such as amphetamine and cocaine occurs because of pH differences between blood (7.4) and oral fluid (6.8 at rest). Free uncharged drug is in equilibrium between blood and oral fluid. At the lower pH in oral fluid, weak bases ionise, increasing total oral fluid drug concentrations. Because most narcotic drugs are basic, they are detected in higher concentrations in oral fluid than in plasma. The presence of THC in oral fluid comes largely from contamination and not portioning of blood to oral fluid. [4–7]

The objective of this article is to compare the number of drivers in Belgium who have drug concentrations above the legal cut-offs for driving under influence of illicit substances in blood and oral fluid, and in addition, to compare the percentage of positives in oral fluid for those who refused to give blood with the respondents who gave both sample types.

Methods

Between January 2008 and September 2009, a roadside survey was conducted in Belgium as part of the European integrated project DRUID (Driving Under the Influence of Drugs, alcohol and medicines). The objective of DRUID is to give scientific support to the European Union transport policy to reach the 2010 road safety target by establishing guidelines and measures to combat impaired driving. Geographic distribution of the roadside sessions was performed systematically: an equal number of sessions was scheduled in the catchment area of each hospital participating in a study of seriously injured drivers. [8,9]

Each volunteer was asked to provide a blood sample (5mL tube with potassium oxalate) and an oral fluid sample collected with the StatSure™ Saliva Sampler ™. The collection device consists of a cellulose pad on a plastic stick. When approximately 1mL sample has been collected, an indicator on the stick turns blue. The stick was then sealed in a tube containing 1mL of buffer. Drivers could refuse to participate in the study, or if they wanted to participate they could refuse to give a blood sample and only fill in a questionnaire and give an oral fluid sample. A total of 2750 drivers provided both a blood and an oral fluid sample, while 199 drivers only provided an oral fluid sample and 3206 persons refused to participate in the survey.

Samples were transported under cooled conditions to the laboratory where the toxicological analyses for 11 illicit psychoactive substances and metabolites were performed.

In oral fluid substances were analysed using liquid-liquid extraction (LLE) followed by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). [10] Tetrahydrocannabinol (THC) in blood samples was initially screened for using enzyme-linked immunoassay (ELISA) and confirmed using LLE followed by gas chromatography-mass spectrometry (GC/MS). [11] Solid phase extraction of blood samples followed by UPLC-MS/MS was used to analyse for the other illicit substances. [9]

The amount of collected oral fluid was determined by weighing the collector. The dilution with buffer was taken into account when calculating analytical results expressed in g/L or ng/mL of undiluted oral fluid. This was calculated using a formula (Eq.2.1)

$$C_{corrected} = \frac{C_{uncorrected}\ x\ (1 + w - \overline{w})}{2\ x\ (w - \overline{w})}$$

$\overline{w}$ = average weight of empty StatSure device

w = weight of sample and StatSure device

$C_{uncorrected}$ = uncorrected concentration of analyte

$C_{corrected}$ = concentration of analyte corrected for volume of oral fluid collected

Eq. 2.1. Weighing procedure for calculating analytical results of undiluted oral fluid

Table 2.1. Cut-offs and blood/plasma ratios of substances recorded in the law

Substance	Belgian cut-off (ng/mL) for the onsite oral fluid test [1]	French cut-off (ng/mL) for the onsite oral fluid test [2]	Belgian cut-off (ng/mL) for oral fluid confir-mation analysis [1]	Belgian cut-off (ng/mL) for plasma analysis [1]	Blood / Plasma ratio [13]	Equivalent Belgian cut-off (ng/mL) for whole blood	SAMSHA confirmation cut-off in oral fluid [4]	Talloires cut-off for blood[4]	AS4760 Australia cut-off in oral fluid[25]	DRUID cut-off oral fluid / blood [14]
THC	25	15	10	1	0.55	0.55	2	1	10	27 / 1
Amphetamine	50	50	25	25	0.65	16.25	50	20	25	360 / 20
MDMA	50	50	25	25	1.0*	25*	50	20	25	270 / 20
Morphine	10	10	5	10	1.02	10.2	40	10	25	95 / 10
6-acetylmorphine	10	10	5	10	0.57	5.7	4	10	10	16 / 10
Cocaine	20	10	10	25	1.0	25	8	10	25	170 / 10
Benzoylecgonine	20	10	10	25	1.0*	25*	8	50	25	95 / 50

* no ratio available in the literature, presumed to be 1

The results of analysis were interpreted as being positive or negative based on the confirmation cut-offs for oral fluid and plasma in the Belgian law (Table 2.1). [1]

As part of the uniform methods used in the DRUID epidemiological studies, whole blood was analysed. [12] A blood/plasma ratio was used to convert the Belgian legal plasma cut-offs into whole blood values. If no ratio was available from the literature, it was presumed to be 1 (Table 2.1). [13]

Percentages of positive findings and concentration ranges were calculated using Microsoft Office Excel 2007. Chi-square and Fisher Exact were calculated with the MedCalc software (Mariakerke, Belgium) and used to determine differences in distribution.

The study protocols were approved by the ethics committee of Ghent University Hospital. The standard protocol complied with recognized standards of human subjects' protection.

Results

Out of the 2750 respondents for which blood and oral fluid samples were available, 39 blood concentrations and 106 oral fluid concentrations were above the cut-off for illicit substances mentioned in the Belgian law (Figure 2.1). Taking into account the number of respondents positive for more than one illicit substance, 28 (1.0%) respondents would be sanctioned for driving under the influence of illicit drugs according to the cut-off in blood, 71 (2.6%) respondents according to the cut-off in oral fluid. This difference is significant ($p<0.01$).

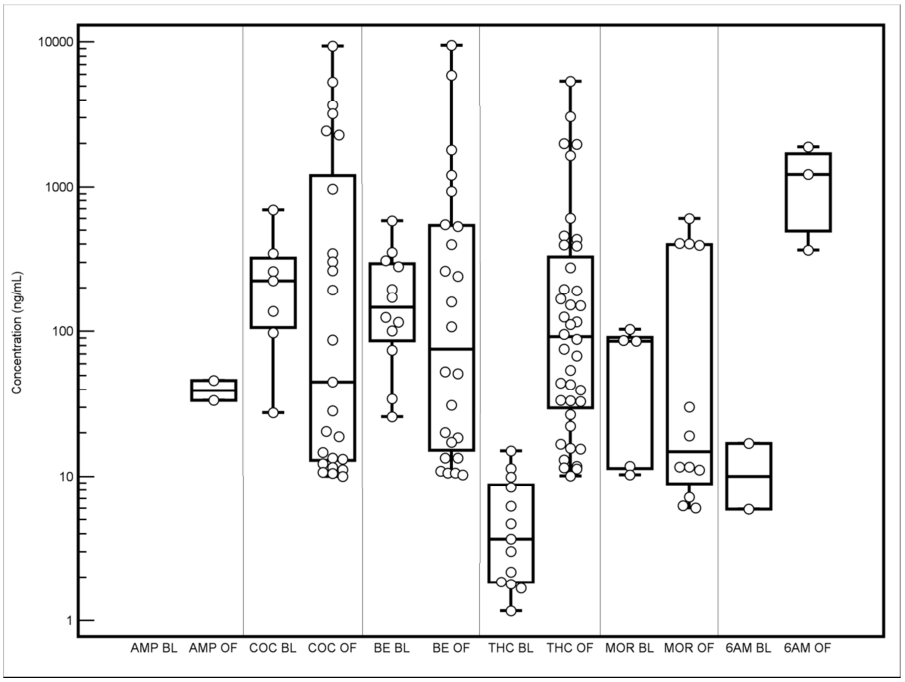

Figure 2.1. Comparison of the concentration (logarithmic scale) above the Belgian cut-offs of six drugs in blood and oral fluid (n= 2750): AMP: amphetamine, COC: cocaine, BE: benzoylecgonine, THC: tetrahydrocannabinol, MOR: morphine, 6AM: 6-acetylmorphine, BL: blood, OF: oral fluid.

Figure 2.1 shows the concentrations of the drugs in blood and oral fluid. A higher number of positives in oral fluid compared to blood was observed for all drugs. The number of positive samples in oral fluid and blood, respectively, was 2 and 0 for amphetamine, 25 and 7 for cocaine, 24 and 12 for benzyolecgonine, 40 and 13 for THC, 12 and 5 for morphine and 3 and 2 for 6-acetylmorphine. The average ratio of positive in oral fluid to positive in blood was 2.5, ranging from 1.5 for 6-acetylmorphine to 3.6 for cocaine.

A second comparison was made in addition. The results on positive oral fluid samples in the group giving both samples was compared to the positive results in the group of those who did not give a blood sample.

Out of the 199 respondents for which only an oral fluid sample was available, 26 (13%) oral fluid concentrations were above the cut-off for illicit substances mentioned in the Belgian law (Table 2.2). Taking into account the number of respondents positive for more than one illicit substance, 15 respondents (7.5%) would be punishable for driving under the influence of illicit drugs. This percentage was significantly (p=0.0005) higher than in the group that also gave a blood sample (2.6%).

No significant difference was found between both groups concerning the gender distribution (p= 0.447), in both groups males were more represented than females. Concerning age, no difference in mean was found between both groups (p=0.242), however when dividing into age groups statistical differences were found for the groups 18-24 y (p= 0.0373) and 35-49 y (p=0.0332). Drivers aged 18 till 24 were found more in the group of drivers who gave only an oral fluid sample, the opposite was observed for drivers aged 35-49.

Table 2.2 gives an overview of the percentage of drivers positive for the different substances included in the Belgian law within each group, together with the p-values of the Fisher exact tests. Statistically significant differences were observed for THC, 6-acetylmorphine, cocaine and benzoylecgonine.

Table 2.20. Comparison of the percentage of positives in oral fluid above the cut-offs in the Belgian Law between the group of drivers who gave only an oral fluid sample and the group who gave both blood and oral fluid samples

Substance	Percentage of positives		P (Fisher exact test)
	Only oral fluid sample available (n= 199)	Oral fluid and blood sample available (n=2750)	
THC	3.52	1.45	0.0359*
Amphetamine	0	0.07	1
MDMA	0.50	0	0.0675
Morphine	1.51	0.44	0.0755
6-acetylmorphine	1.01	0.11	0.0396*
Cocaine	3.52	0.91	0.0006*
Benzoylecgonine	3.02	0.91	0.0155*

THC: Δ^9-tetrahydrocannabinol
* significant difference

Discussion

When using the cut-offs of the Belgian law, 1.0% of the driving population would be punishable for driving under the influence of illicit drugs based on the analysis of blood, 2.9% based on oral fluid analysis. Recent studies in Europe and Australia have shown similar percentage of drivers testing positive for illicit substances. On a European level, illicit drugs are estimated to be used by 1.90% of drivers. [14] Weighted prevalence (see chapter 1, section 1.2.2.1) for amphetamines, cannabis and cocaine was respectively 0.1%, 1.3% and 0.4% (based on oral fluid and blood results, using equivalent cut-offs, see Table 2.1). In Victoria, 2.4% of screened drivers tested positive for cannabis, MDMA or amphetamines. [15] Two studies conducted in Queensland in 2007 and 2009 reported that respectively 3.5% and 3.7% of the included drivers were confirmed positive for at least on illicit substance. [16,17]

The figures show that the Belgian Law is more strict now that confirmation is performed on oral fluid samples rather than in blood.
The group of respondents who refused to give a blood sample turned out to have a higher number of positives. People driving under influence of drugs were probably more reluctant to provide a blood sample in a situation when driving under the influence of drugs can be sanctioned based on the analysis of blood.

Most oral fluid/blood ratios implicate higher concentrations of drugs in oral fluid. Comparing both sets of cut-offs, therefore it is remarkable that the legal limits in oral fluid are equal to or lower than those in plasma (except for THC). Nevertheless the values are in line with recommended oral fluid cut-offs by SAMHSA (Substance Abuse and Mental Health Service Administration), DRUID and the expert group that met at Talloires. [4,18–20]

The legal confirmation cut-offs for oral fluid have been determined in function of the screening cut-offs. The latter were based on the detection limits of existing on-site tests. The screening cut-offs are similar to, or somewhat higher than those in France (Table 2.1), and confirmation cut-offs are also higher than those used in Victoria (2 ng/mL for THC, and 5 ng/mL for methamphetamine and MDMA). [15] The confirmation cut-offs were set at half the screening values. If confirmation cut-offs in oral fluid would be set equivalent to blood (i.e. higher values in oral fluid as stated in the current law), a significant number of false positive screenings would occur, with consequences such as 12-hour withdrawal of the driving license. In addition the law is a zero-tolerance law. Still, some criticism was raised on this legislation, primarily because a substance can still be identified days after consumption, when it is no longer producing psychotropic effects. According to critics, this is no longer related to road safety issues. [21,22] However, the previous legislation included screening in urine, with a much longer detection window, that resulted in approximately 15 % ‘false-positives’ (positive urine screenings that could not be confirmed in blood). [23]

Conclusion

Depending on the cut-offs used, using oral fluid as matrix could increase the number of positives in roadside surveys and enforcement of drugged driving legislation. This will result in detecting more people who drive after consumption of an illicit substance. Many will argue that at the current low cut-offs in oral fluid, the drivers are not under the influence, although for example, there is evidence that many accidents occur in the 'crash' phase long after taking stimulant drugs. [24] In many European countries and US states, there is a zero-tolerance for drugs in traffic. Lower cut-offs in oral fluid might result in more apprehensions.

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CHAPTER 3

Roadside drug testing:

Comparison of two legal approaches in Belgium

To estimate the difference between the enforcement procedure under the old and the new DUI law in Belgium, both legal approaches were compared based on the results of confirmation analysis in plasma. They were derived from the database of the toxicological laboratory of the National Institute for Criminalistics and Criminology, where data on more than 8000 cases were available. The data were derived from routine roadside controls all over Belgium and were fully anonymised.

Quantitative analysis disclosed that fewer false positives were observed since the implementation of the new legislation and that more recent drug use was targeted.

Personal input:

- Statistical processing
- Writing the paper

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Abstract

Background: Internationally, urine on-site testing has been used for detecting drivers under the influence of drugs (DUID) but more and more countries, such as Belgium, are switching to oral fluid screening.

Objective: To compare the previous (published in 1999) and current (published 2009) enforcement procedures of DUID in Belgium. The two evaluated procedures differ in the way the drivers are screened by the police (signs of impairment versus signs of recent drug use), the matrix for screening (urine versus oral fluid) and the analytical cut-off concentrations in plasma.

Methods: Data on positive screening and confirmation results were gathered from 1st April 2008 to 30th September 2010, when urine screening (Dipro Druglab panel test) was performed; and from 1st October 2010 to 31st March 2013, when an on-site oral fluid test (Securetec Drugwipe 5+) was used.

Results: Approximately 4100 data sets related to urine screening and 3900 data sets related to oral fluid screening were studied. Eighty-eight percent of positive urine on-site tests yielded positive results in plasma for cannabis, 21% for cocaine, 20% for amphetamines and 7% for opiates. Sixty-six percent of the positive oral fluid on-site tests yielded positive results in plasma for cannabis, 30% for cocaine, 28% for amphetamines and 8% for opiates. For cannabis, opiates and amphetamines more negative results in plasma were observed in the period of urine screening.

Conclusions: The percentage of plasma samples of tested drivers, in which none of the positive screened target drugs were present in a concentration above the legal cut-off value, has decreased from 17% to 8% since the introduction of the current legislation involving a checklist of external signs of drug use without divided attention tests, oral fluid screening and lower confirmation cut-offs in plasma.

Introduction

Worldwide, the problem of driving under the influence of (il)legal substances is gaining wider attention. The EU funded project DRUID (Driving Under the Influence of Drugs Alcohol and Medicines) has shown that approximately 2% of the European drivers had recently used recreational drugs. [1] Between 28% and 53% of seriously injured drivers tested positive for at least one psychoactive substance (alcohol, recreational or medicinal drugs). [2] In Belgium almost 5% of seriously injured drivers had used a single illicit drug with the highest prevalence for Δ9-tetrahydrocannabinol (THC) (2.5%) whilst 1.5% had combined cannabis use with alcohol. [3]

As a result, driving under the influence of drugs (DUID) has increasingly become a focus for law enforcement. Action against DUID often starts with on-site screening where urine and oral fluid are two matrices that can be used for the on-site tests. With urine, large sample volumes can be obtained and furthermore this body fluid contains large amounts of drug metabolites. Nevertheless, oral fluid has many advantages for on-site testing with no requirement for a restroom whilst same-sex collectors can be used. Furthermore, the collection of oral fluid can be observed and thereby there is a low risk of accidental contamination or deliberate adulteration. In addition, oral fluid better reflects recent drug use in comparison to the drug metabolites in urine (e.g. 11-nor-9-carboxy-Δ9-tetraydrocannabinol, THC-COOH) that can be detected for days or even weeks after the drug was last used. [4,5]

In Belgium, the national institute for road safety (BIVV) reported that the number of roadside DUID controls should increase drastically to create a deterrent effect for drugged drivers. [6] However, the use of a comprehensive test battery including four psychomotor tests followed by the application of a screening test based on urine was considered to be too long and thereby impractical. [7] Furthermore, scientific observations suggest that oral fluid results are superior when compared to urine in correlating with impairment symptoms. These factors led to a change in legislation in Belgium with a new law becoming effective in October 2010. [8] After an initial selection on the basis of a limited field sobriety test (checklist for external signs of recent use), screening is performed on oral fluid and then positives can be confirmed using plasma or oral fluid, with lower cut-off values for plasma in comparison to the previous legislation. In practice, since the oral fluid collection system is not yet specified, the confirmation analysis has been performed on plasma since the change in legislation. [9]

Figure 3.1 describes the enforcement procedures under both legal approaches.

The objective of this publication is to compare both legal approaches in terms of the percentages of ‘false positives’. A false positive result is defined as a positive roadside screening test that is not confirmed by a positive plasma analysis for the target drugs, taking the legal cut-off values into account. In addition, the paper includes an overview of screening tests not confirmed by plasma analysis using both sets of cut-offs (previous vs current legislation).

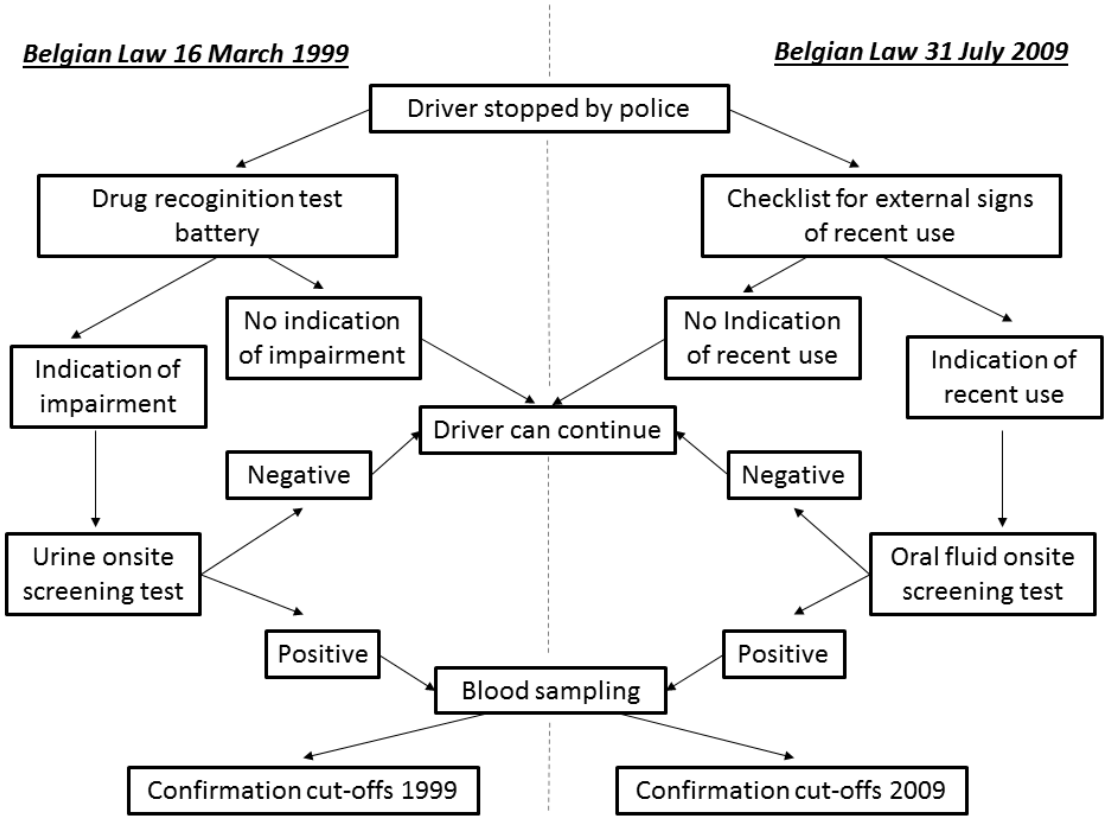

Figure 3.1. Flow chart of both legal procedures on DUID.

Methods

Analysis data were gathered within two adjacent periods of time (2.5 year each): a) from 1st April 2008 to 30th September 2010, during which urine screening was performed; b) from 1st October 2010 to 31st March 2013, during which an on-site oral fluid test was used. This comparison was not based on paired samples of urine, oral fluid and blood.

Test battery

The standardized test battery used in the legal approach of 1999 consisted of two parts, namely observation of physical signs and 'attention distributive tests'. [10] The most important physical signs were the appearance of the eyes (e.g. shiny glow, irregular reaction of the pupils, blurred eyes) and trembling/shivering of body parts. The 'attention distributive tests' comprised of four tests: 'Romberg's test', 'one leg stand', 'walk and turn' and 'finger-to-nose', The result of the test battery was positive when at least one physical sign and at least one erratically performed attention distributive test could be noted.

The test battery was time consuming and was used to detect impairment. Therefore the project ROPS (driving under influence of psychoactive substances) recommended the implementation of a less time consuming checklist, which evaluates external signs to detect recent use. [7]

A new standardized checklist to determine indications of signs of recent drug use in traffic was published in a royal decree on 17th September 2010. [11] At least 3 signs, divided over at least 2 different categories, must be identified to obtain a positive result. The categories are: eyes (e.g. shiny, weepy, blurred, bloodshot, narrow pupils), face (e.g. dry lips, grinding teeth, repeated snorting), behavior (e.g. nervous/agitated, aggressiveness, mental confusion), state of mind (e.g. euphoria, unstable mood), language (e.g. stammering, repeating words, verbosity), walk (e.g. disturbance of equilibrium) and others (e.g. trembling limbs, sweating, fast or slow reflexes).

On-site screening devices

Under the previous legislation the Dipro Druglab® panel test (DiproMed, Weigelsdorf, Germany), an immunological assay which detects the presence of metabolites of cannabinoids, amphetamines, cocaine and opiates in urine, was used. It consists of a test element and a urine recipient. The absorbing strips of the test kit are dipped in the sample until a pink color is visible in the test windows (this takes ± 30 seconds at ambient temperature). The test is then placed on a flat surface. Results can be read after 3 to 8 minutes. A test is valid when a red line is visible in the control zone. The absence of a red line in the test zone indicates a positive result.

With the current legislation the Securetec Drugwipe-5+® device (Securetec, Munich, Germany), an immunoassay which screens for cannabinoids, amphetamines, cocaine and opiates in oral fluid, was used. The sample applicator of the device consists of two small pads that collect oral fluid (about 10 – 20 µL) by wiping the tongue and cheeks. Once the sample applicator is fixed onto the test strip, the test is held vertically and an integrated buffer ampoule is broken. After 15 s, the test is placed

horizontally and the results are visible within 10 minutes. A test is valid when a red line is visible in the control zone. The appearance of a red line in the test zone indicates a positive result.

Table 3.1 gives an overview of the screening cut-off values of the two devices, as stated by the manufacturers, and the legal cut-off values for the confirmation analysis.

Table 3.121. Cut-offs (µg/L) for onsite tests and confirmation analysis

Substance	**Dipro-Druglab ® panel test (urine)**		**Securetec Drugwipe-5+® (oral fluid)**		**Confirmation cut-offs [8][9]**			
	Target	Cut-off	Target	Cut-off	Target	Plasma (1999 law)	Plasma (2009 law)	Oral fluid (2009 law
Amphetamine	d-amphetamine	1000	d-amphetamine	50	Amphetamine	50	25	25
Meth-amphetamine	d-methamphetamine	1000	d-methamphetamine	25	Not in scope			
MDMA	(Meth)amphetamine*	2000	MDMA	25	MDMA	50**	25	25
Cocaine	Benzoylecgonine	300	Benzoylecgonine	25	Cocaine	50	25	10
					Benzoylecgonine	50	25	10
Opiates	Morphine	300	Morphine	10	Morphine	20	10	5
					6-acetylmorphine	n.a.	n.a.	5
Cannabis	THCCOOH	50	THC	25	THC	2	1	10

* Cross-reactivity of MDMA with the (meth)amphetamine target
** also for MDEA and MBDB
MDMA= 3,4-methylenedioxymethamphetamine, THC= Δ9-tetrahydrocannabinol, THCCOOH= 11-nor-9-carboxy-Δ9-tetraydrocannabinol
N.a.: not applicable

Confirmation analysis

Blood samples were collected in three blood tubes using sodium fluoride as stabilizer and potassium oxalate as anticoagulant. They were cooled (4°C) and centrifuged. The plasma was frozen at -20°C until analysis at the laboratory of toxicology of the National Institute of Criminalistics and Criminology. The plasma was analyzed with GC-MS for morphine, codeine, benzoylecgonine, methylecgonine, cocaine, amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxy-N-ethylamphetamine (MDEA), N-methyl-1,3-benzodioxolylbutanamine (MBDB), Δ9-tetrahydrocannabinol (THC), 11-Hydroxy-Δ9-tetrahydrocannabinol (OH-THC) and 11-nor-9-carboxy-Δ9-tetraydrocannabinol (THC-COOH). The methods used were previously published. [12–15] In 2011 the analysis of THC was switched to UPLC-MS/MS, and THC-COOH and OH-THC were left out of the scope. [16]

Plasma samples of subjects with a positive screening result for opiates that could not be confirmed for morphine, were screened with an UPLC-MS/MS method[17] for the presence of other opiates and opioids: buprenorphine, norbuprenorphine, 6-acetylmorphine, codeine, hydromorphone, hydrocodone, norcodeine, pholcodine, oxycodone, oxymorphone, dihydrocodeine, ethylmorphine, methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), fentanyl, norfentanyl, meperidine, normeperidine, tramadol, pentazocine and propoxyphene. These extra analyses were performed for this study, to investigate potential cross-reactions. In the case of an authentic DUID-sample, only the substances mentioned in the legislation will be reported. So the presence of one of these substances does not have legal consequences for a driver.

Table 3.1 shows the cut-offs that were applied in each testing period. [8,9]

Data analysis

Percentages of positive findings were calculated using Microsoft Office Excel 2010. Statistical analysis was made using IBM SPSS Statistics 21. Chi-square tests were performed using a web-based tool on contingency tables on www.vassarstats.net. Mann Whitney U tests were performed to compare the distribution of drug concentrations in the plasma samples under the previous or current legislation.
Since blood samples were either taken after a positive on-site test, or when screening was not performed (no test available, refusal of driver, etc.), no data on negative screenings was available. Only the diagnostic values 'True Positive' and 'False Positive' could be calculated:

True Positives (TP) are the cases with positive screening results and positive confirmation analyses. False Positives (FP) are the cases with positive screening results and negative confirmation analyses.

Box and whisker plots were drawn (one for each analyte mentioned in the law), to show the distribution of the non-zero concentrations in plasma by screening procedure. The box in these box and whisker plots represents those cases between the 75th and 25th percentile (Q3-Q1), whilst the line that bisects the box is the median concentration for the cases. The whiskers that protrude from the box extend to 1.5 times "Q3-Q1" or, if no case has a value in that range, to the minimum or maximum values. If the data are distributed normally, approximately 95% of the cases are expected to lie between the whiskers. Outliers, denoted by a point, are defined as cases that do not fall within the whiskers. Extreme outliers are denoted by asterisks and represent cases that have values more than three times "Q3-Q1" beyond the limits of the box.

Results

Comparison of the positive screening results during previous and current legislation

In total 4109 datasets related to the previous legislation and 3917 datasets related to the current legislation were analyzed.

Of the approximately 4100 urine screening results, 21% screened positive for cocaine, 88% for cannabis, 7% for opiates and 20% for amphetamines.

Of the approximately 3900 oral fluid screening results, 30% screened positive for cocaine, 66% for cannabis, 8% for opiates and 28% for amphetamines.

The on-site tests were positive for only one drug class in 70% (urine) and 72% (oral fluid) cases, respectively. Two drug classes were observed in approximately 24% of tests in both types of screening. Positive tests for three drug classes were observed in 5.4% (urine) and 3.9% (oral fluid), and four drug classes in 0.3% (urine) and 0.4% (oral fluid).

The most common combinations were cocaine and cannabis (10% in urine screening and 8% in oral fluid screening) and amphetamine and cannabis (9% in urine screening and 8% in oral fluid screening).

Table 3.2 gives an overview of the screening and confirmation results for both legal approaches. Chi-square tests indicate a difference in false positives between both legislations for all substances except cocaine.

Table 3.22. Overview of positive screening results with respect to the confirmation analysis, taking the legal cut-off values into account.

	Previous legislation (1999)			**Current legislation (2009)**			**Statistics**	
	Total positive screenings (urine)	True Positive	False Positive	Total positive screenings (oral fluid)	True Positive	False Positive	Chi^2	p-value
Cannabinoids1	3615	75.2%	24.8%	2600	91.4%	8.6%	269.82	<0.001
Cocaine[2]	858	63.8%	36.2%	1163	64.0%	36.0%	0	1
Amphetamines[3]	825	69.3%	30.7%	1106	74.4%	25.6%	5.71	0.0169
Opiates	292	28.1%	71.9%	317	47.3%	52.7%	23.04	<0.001
Total results[4]	5590	70.1%	29.9%	5186	78.9%	21.1%		

[1] 2 missing confirmation results within group of oral fluid screening
[2] 1 missing confirmation result within group of urine screening
[3] 2 missing confirmation results within group of oral fluid screening
[4] total number of screening results, an onsite test can be positive for more than one substance group

During the previous legislation 17% of the tested drivers revealed a negative plasma result or a plasma concentration below the legal cut-off value for the positively screened target drugs. Since the

application of the current legislation, the percentage of drivers in which a positive oral fluid test was not associated to a plasma result above the legal cut-off for any of the positively screened target drugs decreased to 8%.

For cannabis, fewer false positive results were observed since the current legislation was implemented.

In 86% of the positive urine screenings, THC could be detected above the limit of quantification (LOQ) in the corresponding plasma sample. In the dataset with oral fluid screening, 93% of the plasma samples were positive for THC (Chi^2= 90.99, p<0.001).

Higher plasma THC concentrations could be observed (Z= -10.9, p<0.001) after oral fluid screening (median concentration of 8.5 ng/mL for oral fluid screening vs 6.0 ng/mL for urine screening) (see Figure 3.2).

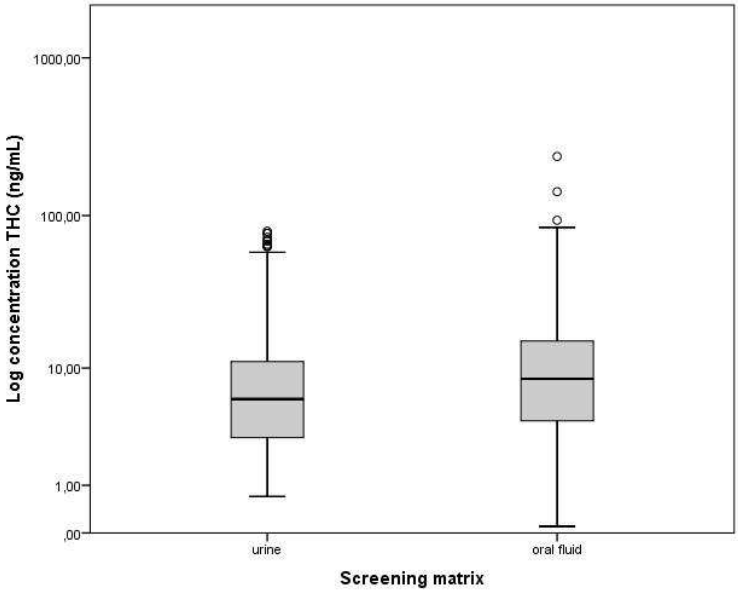

Figure 3.2. Distribution of plasma concentrations (Log scale, non-zero) of THC with respect to the screening matrix

For cocaine, there was no change in the percentage of false positive results since the introduction of the current legislation (see Table 3.2).

In 84% of the positive urine screenings, cocaine and/or benzoylecgonine were detected above the LOQ in the corresponding plasma samples. Fifty-five percent of these samples contained both benzoylecgonine and cocaine, while 45% of the samples only contained benzoylecgonine. In 73% of the positive oral fluid screenings, cocaine and/or benzoylecgonine were detected in the corresponding

plasma samples, but of these 65% contained both benzoylecgonine and cocaine and in 35% of the samples only benzoylecgonine was detected. A difference between both legal approaches was found (Chi^2=16.32, p<0.001) with a higher percentage of plasma samples that contained both analytes since the implementation of the oral fluid on-site test.

Higher plasma benzoylecgonine concentrations could be observed (Z= -3.532, p<0.001) after oral fluid screening (median concentration of 273 ng/mL for oral fluid screening vs 197 ng/mL for urine screening) (see Figure 3.3).

For cocaine no difference was found (Z= -0.801, p= 0.423) (median concentrations of 46.5 ng/ml for oral fluid screening and 44 ng/mL for urine screening) (see Figure 3.3).

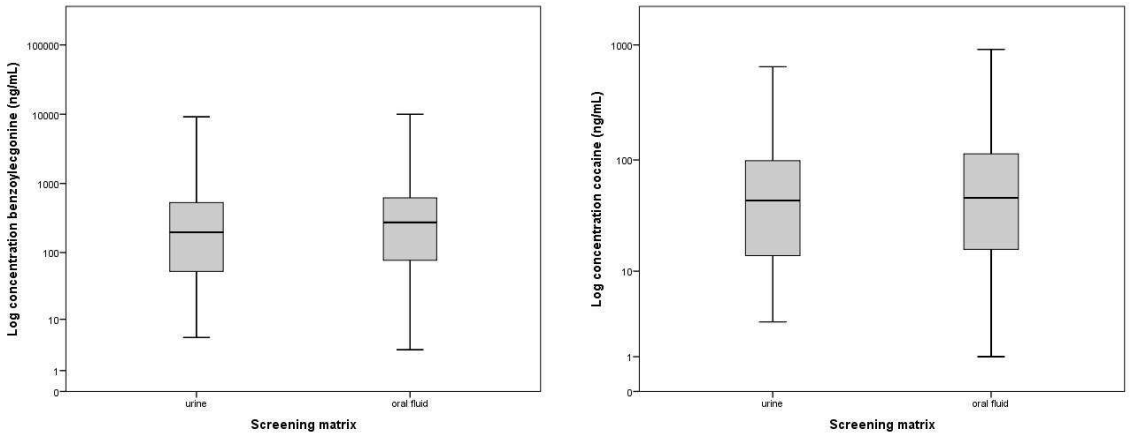

Figure 3.3. Distribution of plasma concentrations (log scale, non-zero) for benzoylecgonine and cocaine with respect to the screening matrix. A significant difference was observed for benzoylecgonine.

For amphetamines, fewer false positives were observed since the implementation of the current legislation.

In 90% of the positive urine screenings, MDMA and/or amphetamine could be detected above the LOQ in the corresponding plasma. In the dataset with oral fluid screening, 78% of the plasma samples was positive for MDMA and/or amphetamine (Chi^2= 44.35, p<0.001).

Of the true positives in urine, 12.1% confirmed positive for MDMA only in plasma, 80.6% for amphetamine only, and 7.3% for both substances. For oral fluid screening, these percentages were 14.0%, 76.0% and 10.0%, respectively. For three urine screenings and one oral fluid screening methamphetamine was detected in the plasma.

When comparing the distribution of amphetamine and MDMA concentrations in the plasma samples associated to either urine or oral fluid screening, a difference could be observed for amphetamine (Z= -9,333, p<0.001) with higher concentrations in plasma since screening was performed in oral fluid

(median plasma concentrations: 258 vs 124 ng/mL). For MDMA no difference was found (Z= -0.579, p= 0.562) (median plasma concentrations: 178 and 166 ng/mL) (see Figure 3.4).

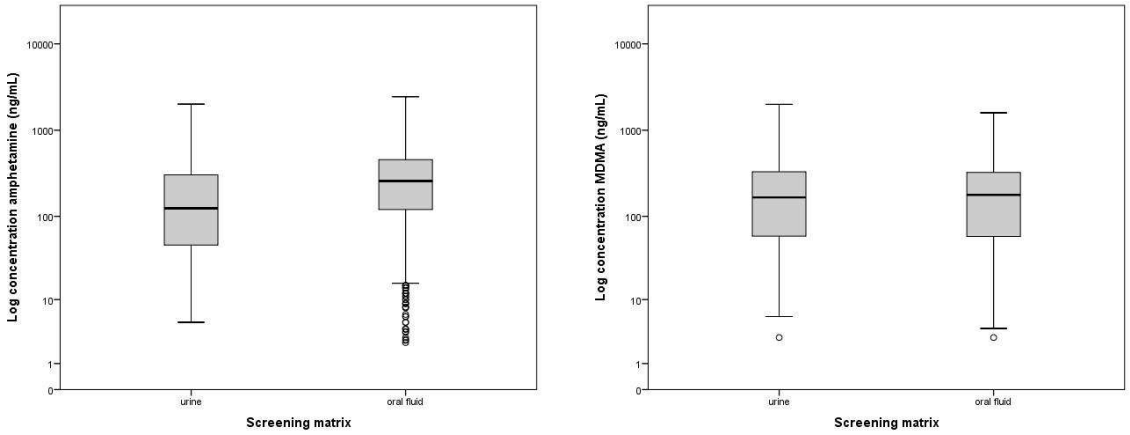

Figure 3.4. Distribution of plasma concentrations (log scale, non-zero) for amphetamine and MDMA with respect to both screening matrices. A significant difference was observed for amphetamine.

For opiates, more true positive results were observed under the current legislation.

In respectively 38% and 56 % of the positive urine screenings and oral fluid on-site tests, morphine could be detected above the LOQ in the corresponding plasma (Chi^2= 17.93, p<0.001).

Table 3.3 gives an overview of the results for the extra analysis on plasma samples. A significant difference between both time periods could be observed for oxycodone.

Table 3.323. Results for the extra UPLC-MSMS analysis on the plasma samples for which false positive opiate screening was observed

Analyte	Urine screening (n=210)	Oral fluid screening (n=167)	Chi^2 (p-value)
Morphine	30 (14.3%)	27 (16.2%)	0.13 (0.72)
Codeine	13 (6.2%)	20 (12.0%)	3.21 (0.07)
Methadone	32 (15.2%)	35 (21.0%)	1.71 (0.19)
Pholcodine	18 (8.6%)	6 (3.6%)	3.08 (0.08)
Oxycodone	4 (1.9%)	22 (13.2%)	16.68 (<0.001)*
Tramadol	3 (1.4%)	3 (1.8%)	Fisher test (1.00)
Fentanyl		1 (0.6%)	

*significant difference

No difference could be found in the distribution of morphine concentrations in plasma samples associated to either urine or oral fluid screening (Z= -0.726, p= 0.468) (median plasma concentrations: 35 and 30 ng/mL).

False positives with previous and current plasma cut-offs

Table 3.4 gives an overview of the screening tests not confirmed by plasma analysis using the old cut-offs (previous legislation) and the new cut-offs (current legislation). Comparisons were made for urine versus oral fluid screening with the old cut-offs on the one hand, and, on the other hand, urine versus oral fluid screening with the new cut-offs.

Table 3.4. False positive results with application of the previous and current cut-off values for plasma. *indicates a significant difference

	Cocaine	Cannabinoids	Opiates	Amphetamines
Old cut-off (1999 law)				
Urine	36.2%	24.8%	71.9%	30.7%
Oral fluid	40.8%	15.2%	61.5%	29.1%
Chi² (p-value)	4.2 (0.040)*	84.4 (<0.001)*	6.9 (0.009)*	0.47 (0.493)
New cut-off (2009 law)				
Urine	29.3%	14.5%	61.6%	20.6%
Oral fluid	36.0%	8.6%	52.7%	25.6%
Chi² (p-value)	9.9 (0.002)*	48.8 (<0.001)*	4.6 (0.032)*	6.3 (0.012)*

When using the old confirmation cut-offs, fewer false positives were observed for cannabinoids and opiates when using an oral fluid on-site test, however for cocaine fewer false positives were seen when using urine screening.

When using the new cut-offs fewer false positives for cannabinoids and opiates were seen when using oral fluid screening, while more false positives could be observed for cocaine and amphetamines.

Discussion

When enforcing DUID legislation, false positive screening results have to be avoided, since (in Belgium) a positive screening test results immediately in a driving ban of 12 hours for the DUID suspected driver, even when the confirmation analysis states negative, leading to unnecessary financial consequences for the judicial authorities. The comparison of the two legal approaches demonstrates that the percentage of false positive screening results has decreased since the introduction of the new legislation, i.e. a fast and limited field sobriety test followed by an oral fluid screening and the lowering of the cut-offs for confirmation in plasma. The percentage of plasma samples of tested drivers, in which none of the positive screened target drugs were present in a

concentration above the legal cut-off value, has decreased from 17% to 8% since the introduction of the current legislation.

For cannabinoids, the increase of true positives as a result of the introduction of the new legal procedure is significant. Since screening is performed in oral fluid, a significantly higher median plasma concentration for THC is observed, indicating that the new procedure targets recent use to a higher extent than before. This can be explained by the fact that the detection time of THC in oral fluid is more similar to the detection window of THC in blood when compared to that of the metabolite THC-COOH in urine. The high number of false positive urine screenings, for which only THC-COOH was detected in plasma, suggests that the subjects had used cannabis at some time before being tested.
The decrease in false positives following the introduction of the new approach is mostly due to the change in the screening procedure. Since cannabis is the most prevalent illicit drug detected in Belgian drivers (e.g. in 2013 approximately 60% of the analyzed blood samples were found to be positive for THC), the benefits of the new legal approach in the case of cannabis use should not be underestimated.
Based on the confidential information on cross-reactivity for THC shared by the manufacturers, and the concentration values found that can be expected for the cross-reacting drugs based on data in the literature, [18,19] no (false) positive results are to be expected.

Although the percentage of true positives for cocaine did not increase with the new legal approach, the number of cases where both cocaine and its metabolite benzoylecgonine were detected in plasma, was higher when oral fluid screening was performed compared to urine screening. In addition, the median benzoylecgonine concentration in plasma was significantly higher with oral fluid screening. This suggests that screening in oral fluid detects more recent cocaine use. The comparison of the false positives according to the previous and current cut-offs shows that the use of oral fluid on-site tests tends to result in more positive screenings that could not be confirmed in plasma. However, as indicated in Table 4, the use of lower confirmation cut-offs corrects for this increase.

The decrease in false positive amphetamine results is mostly achieved by lowering the confirmation cut-offs as observed in Table 3.4. However, the median amphetamine concentration in plasma samples after oral fluid screening is much higher than that after urine screening, indicating that more recent use is detected with oral fluid on-site tests. A possible explanation of the high number of false positives could be the higher concentrations and the longer detection window of basic drugs in urine as well as oral fluid compared to plasma. A second plausible explanation is cross-reactivity. Considering the cross-reactivity data and published concentrations, a (false) positive result could be expected if sufficiently high concentrations of p-methoxyamphetamine (PMA), methylenedioxyamphetamine (MDA), methylenedioxyethylamphetamine (MDEA), phentermine or mephedrone are present in the oral fluid or urine sample. All positive amphetamine screenings were also tested for MDA, MDEA (stated to have cross-reactivity of 10 and 150 µg/L in oral fluid) and MBDB, but they were negative for these substances.

It also has to be noted that the difference in percentage of MDMA confirmation in plasma between both screening matrices (19% vs 24%) could be explained by the fact that MDMA had almost disappeared from the Belgium illicit drug market in the period 2008-2009 due to a shortage in the supply of the precursor.

The high number of false positives for opiates is probably partly due to cross-reactivity. Based on cross-reactivity data, and the concentration values found in the literature, [18] a (false) positive result could for instance be expected if sufficiently high concentrations of codeine, heroin, dihydrocodeine or 6-monoacetylmorphine are present in oral fluid.

The investigation of the blood samples with the false positive on-site tests revealed only a few positives for other opiate(-like) molecules that could have cross-reactivity with the opiate screening tests. Only methadone (which does not cross-react) was found in 15% (previous legislation) and 21% (current legislation) of the false positive cases. The decrease in false positives since the current legislation was introduced tends to be mostly due to the shorter window of detection with oral fluid screening.

It is worth noting that the total number of positive on-site screening tests has not increased, which was, in fact, the objective of the new legislation. This could be due to start-up issues. In the first 3 months of enforcement, an average of 150 samples per month was sent to the laboratory for confirmation. This number decreased to 109 in 2011 and then increased to 137 in 2012. In 2013 an average of 165 confirmation analyses were performed per month. This suggests an enthusiastic start that diminished for a certain period and then increased again in 2013.

Personal communication with police forces has revealed that, although no detailed data set is available on the total number of on-site tests carried out, police officers are now performing more on-site tests than previously, when screening was performed on urine samples. One hypothesis is that due to the execution of a comprehensive test battery, which took a trained police officer up to 45 minutes, a urine test was only performed when impairment was demonstrated. In the current procedure, the checklist for external signs of recent use can be applied in 10-15 minutes. In these cases the pre-selection of drivers takes less time, but is perhaps less effective (certainly for cocaine and amphetamine use). More on-site tests are performed, but the percentage of negative results is higher. This trend can also be observed in the data of the federal motorway police in the Belgian National report published by the EMCDDA (European Monitoring Center for Drugs and Drugs Addiction) (Figure 3.5). Police training is thus of utmost importance.

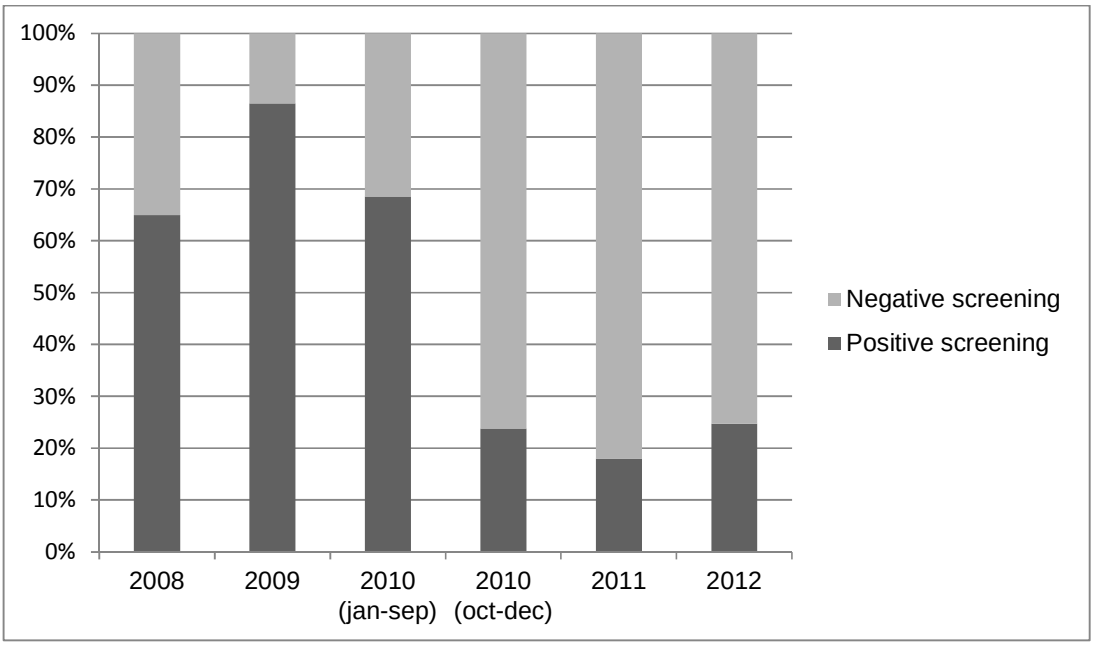

Figure 3.5. Percentage of positive and negative screenings performed by the federal police on motorways.

The decrease in the total number of positive screening tests is largely caused by a lower number of positive screenings for cannabinoids. For all other substance groups, the number of positive screenings is increased. The detection window is different for both screening matrices, certainly in the case of cannabinoids, as discussed above. In addition, the cut-off values for the on-site tests are not equivalent one to another. The screening cut-offs of the Drugwipe are of the same order of magnitude as the confirmation cut-offs in plasma, which could create false positive results as the oral fluid concentrations are often higher than plasma due to the occurrence of ion trapping of basic drugs in oral fluid. [5,20,21] . Although there is a relatively good correlation between the kinetics for several psychoactive substances in blood and oral fluid, no conversion factors have yet been established. In the future both screening and confirmation will be based on oral fluid, with lower confirmation cut-offs, probably resulting in fewer false positives, in particular for cocaine and amphetamines.

A drawback of the current study is the lack of data on negative screening results. Therefore, no true and false negative data could be calculated, indicating that no clear and full view on these parameters of both screening tests could be studied.

Conclusion

By changing the drug screening procedure and lowering the cut-off values for confirmation in plasma, the new law in Belgium has resulted in a better approach towards driving under the influence of drugs. Even with a limited pre-selection of drivers, the screening results for most substance groups resulted in a lower percentage of false positive results. The number of drivers where none of the positively screened target drugs could be confirmed above the legal cut-off value has dropped significantly and our data suggests that more recent drug use is being detected. This trend is undoubtedly demonstrated for cannabis, which is the most widely used illicit drug in Belgium and worldwide.

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CHAPTER 4

Comparison of drug concentrations measured in roadside surveys and in seriously injured drivers in Belgium

To estimate the difference in drug concentrations in injured drivers and roadside respondents we used the confirmation data in blood of both study populations. The data of the Belgian roadside survey and seriously injured drivers study, part of the DRUID WP2, were used for this comparison.

We observed higher amphetamine and benzoylecgonine concentrations in injured drivers. In addition a trend towards higher concentrations of benzodiazepines and Z-drugs was observed.

Personal input:

- Part of data collection at roadside surveys
- Toxicological analyses
    - 5-10% oral fluid analyses using UPLC/MSMS for detection of (il)licit drugs
    - 10-15% of alcohol analyses on blood and oral fluid samples using an enzymatic method
    - ELISA and GCMS analyses on blood samples for detection of THC and THCCOOH
- Statistical processing
- Writing the paper

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Abstract

The objective of this paper is to compare concentrations of alcohol, illicit and medicinal drugs in seriously injured drivers and drivers selected randomly at the roadside.

Blood samples were analysed for alcohol, 17 medicinal drugs and 8 illicit psychoactive substances and/or their metabolites by UPLC-MS/MS and GC/MS in injured drivers admitted to the emergency departments of five hospitals in Belgium between January 2008 and May 2010 and in drivers randomly selected between January 2008 and September 2009.

377 seriously injured drivers and 2750 roadside respondents were selected. In the roadside survey, out of the 203 concentrations above DRUID cut-offs for medicinal drugs, 51% were in the therapeutic range, 46% infratherapeutic and 2.5% supratherapeutic. In the seriously injured drivers, out of the 78 concentrations above DRUID cut-offs for medicinal drugs, these percentages were respectively 63%, 33% and 4%. Significant differences were found in the distribution of concentrations for opioids, benzodiazepines and Z-drugs. For the latter, while in the seriously injured drivers study most concentrations were therapeutic, in the roadside survey most were infratherapeutic. The opposite was observed for the opioids.

Eight and forty-one percent of the roadside respondents and injured drivers respectively had an alcohol concentration above 0.1 g/L, with higher concentrations found in the injured drivers.

For illicit drugs, significant differences were found for amphetamine and cocaine, for which respectively lower and higher concentrations were observed in the blood samples taken in the roadside survey.

Introduction

In 2008-2009 a roadside survey was conducted in Belgium as part of the European integrated project DRUID (Driving Under the Influence of Drugs, alcohol and medicines) to determine the prevalence of alcohol, psychoactive medicinal drugs and illegal drugs among drivers. In 2008-2010 a study on the presence of alcohol and other drugs in drivers admitted to the emergency departments of five hospitals was undertaken within the same European project, in order to investigate the presence of these psychoactive substances among the Belgian injured drivers and the characteristics of the patients involved. [1,2]

While the percentages of drivers in whom alcohol and drugs were detected have been extensively studied and reported, [3–6] little attention has been given to the blood concentrations of the drugs in the two different populations. The objective of this article is to compare the concentrations of medicinal drugs, alcohol and illicit psychoactive substances in blood sampled from seriously injured drivers and respondents of the roadside survey. Our expectation is that higher concentrations will be found in injured drivers than in randomly selected drivers. To our knowledge, this is the first study that compares blood concentrations for drugs in randomly selected drivers at the roadside.

Methods

Data collection

Samples from seriously injured drivers were collected between January 2008 and May 2010 from patients admitted to the emergency departments of Ghent University Hospital, Namur Regional Hospital, Sart Tilman University Hospital (Liège), Leuven University Hospital and Brussels University Hospital. These 5 hospitals were selected because they participated in the 'Belgium Toxicology and Trauma Study' in 1995. [7]

Patients were included in the study when they were drivers of a motor vehicle or a bicycle, aged more than 17 years and when they were admitted because of traumatological reasons (Maximum Abbreviated Injury Score (MAIS) of 2 or higher). [8] A maximum delay of 3 hours between trauma and blood sampling was allowed.

For this analysis, only drivers of a personal car or van were considered, leaving a total of 377 cases.

Roadside sampling was conducted between January 2008 and September 2009. The geographic distribution of the roadside sessions was performed systematically: sessions were scheduled in the catchment area of the hospitals participating in the seriously injured drivers study.

Only car or van drivers who gave a blood sample were included, leaving a total of 2750 roadside respondents.

The study protocols were approved by the ethics committee of Ghent University Hospital.

Toxicology

Blood samples were taken using a 5 mL glass collection tube containing potassium oxalate and sodium fluoride. Samples were stored frozen at the hospitals. Shipments of samples (under cooled conditions) to the laboratory where toxicological analyses were performed took place regularly.

The analyses included determination of ethanol, 17 medicinal and 8 illicit psychoactive substances (see Table 4.1).

Quantitative determination of drug concentrations was carried out on whole blood samples. Liquid-liquid or solid phase extraction procedures were used. Chromatographic separation was performed by gas chromatography (GC) or Ultra Performance Liquid Chromatography (UPLC). Detection was performed by mass spectrometry (MS) or tandem mass spectrometry (MS/MS). Ethanol was quantified using an enzymatic method. Methods have been described in detail elsewhere. [2]

Only concentrations above DRUID cut-offs (see Table 4.1) were included in this study. [1,2] Medications administered after trauma and before blood sampling according to hospital records were not taken into account.

To make a general comparison between injured drivers and respondents of the roadside survey, medicinal drugs were grouped and therapeutic ranges were used to compare the two populations.

Since therapeutic ranges were only available for serum/plasma concentrations, [9] a blood/plasma ratio[10–13] was used to convert the therapeutic values into whole blood concentrations. If no ratio was available, this was presumed to be 1 (Table 4.2).

Data analysis

Percentages of positive findings and concentration ranges were calculated using Microsoft Office Excel 2007. Chi-square and Fisher Exact calculated with the MedCalc software (Mariakerke, Belgium) were used to determine differences in distribution. Mann Whitney tests were performed using the statistical software Stata®.

Results

Table 4.1 gives an overview of the concentration distribution of the substances by group of respondents and the results of the Mann Whitney tests.

Alcohol

In the roadside survey 217 concentrations at or above 0.1 g/L were found for ethanol (range 0.1-2.4 g/L). In the hospital study 156 drivers had an ethanol concentration ranging from 0.1 to 4.0 g/L. Comparing the concentration distribution of ethanol in the two populations, a significant difference was found ($p<0.001$) with much higher concentrations in the injured drivers.

Illicit drugs

Significant differences were found for amphetamine ($p=0.037$) and cocaine ($p=0.011$). Higher amphetamine concentrations were observed in injured drivers. However, only 2 amphetamine concentrations above DRUID cut-off were observed in the roadside survey so the data should be interpreted cautiously. While both concentrations in the roadside survey were below the legal limit in Belgium (25 ng/mL plasma, approximately equivalent to 16 ng/mL in whole blood), all concentrations in the injured drivers were above this cut-off. Cocaine concentrations appeared to be higher in roadside survey drivers; however this result may have been affected by the time delay between accident and sampling in the injured drivers (up to three hours). In fact concentrations of benzoylecgonine, the major metabolite of cocaine, were higher in injured drivers, suggesting the possibility of higher cocaine levels at an earlier time. For THC there was no significant difference. No comparison was performed for 6-acetylmorphine, MDA and MDMA due to the fact that concentrations above DRUID cut-off were not observed or observed only once in one or both populations.

Medicinal drugs

Higher concentrations were observed in the injured drivers compared to the roadside population for lorazepam ($p=0.045$), nordiazepam ($p=0.006$) and zolpidem ($p=0.009$).

There was a trend for methadone ($p=0.053$), mirtazapine ($p=0.053$) and tramadol ($p=0.058$), indicating a possible higher concentration of mirtazapine and tramadol in the injured drivers and higher concentration of methadone in the roadside group.

No comparison could be performed for alprazolam, amitriptyline, clonazepam and flunitrazepam because of insufficient data.

In the roadside survey, out of 203 concentrations for medicinal drugs (morphine excluded, because of the possibility of being the result of heroin use), 104 (51%) lay in the therapeutic range, 94 (46%) were infratherapeutic and 5 (3%) supratherapeutic.

In the hospital study, out of 78 concentrations for medicinal drugs (morphine excluded), 49 (63%) lay in the therapeutic range, 26 (33%) under and 3 (4%) above.
No statistically significant difference was found in the distribution of both groups by therapeutic range (Fisher exact test, p= 0.124).

Figure 4.1 shows the distribution of the concentrations above DRUID cut-off by medicinal drug groups.

Benzodiazepines and Z-drugs

In the roadside survey, more than 36% of benzodiazepine and Z-drugs concentrations were within the therapeutic range. In injured drivers this percentage was almost two times higher (67%).

Significant differences between the two populations were found for the therapeutic range classes (Fisher exact test, p= 0.004). While in the roadside population more samples had concentrations lower than the therapeutic range (p= 0.005), in the hospital study more concentrations fell in the therapeutic range (p= 0.002). Concentrations above the therapeutic range were equally distributed in both studies (p=0.77). This illustrates the trend toward higher concentrations of benzodiazepines and Z-drugs in injured drivers.

Opiates and opioids (codeine, methadone and tramadol)

In the hospital study, 40% of the concentrations were in the therapeutic range. In the roadside survey this percentage was more than two times higher (87%, Fisher exact test, p= 0.008).

Concentrations under the therapeutic range were seen more often in the injured drivers (p= 0.048), while concentrations in the therapeutic range were seen more in the roadside survey (p= 0.006). Supratherapeutic concentrations were equally distributed in both studies (Fisher exact test, p=0.150). For this class of drugs, there was no indication of higher drug concentrations in injured drivers.

Antidepressants

In the roadside survey, 48% of non-zero concentrations were subtherapeutic and 52% were therapeutic. In the hospital study these percentages were 35% and 65% respectively (X^2= 0.75, p= 0.39).

Table 4.1. Distribution of drug concentrations above Druid cut-off. Drivers in roadside survey vs. seriously injured drivers.

Substance	Druid cut-off (ng/mL)	Roadside				Injured drivers				Mann Whitney	p-value	Significant difference
		N	range	mean	median	n	range	mean	median			
6-Acetyl-morphine	10	1	17			0				n.a.	n.a.	no
Alprazolam	10	12	10-54	22	15	0				n.a.	n.a.	no
Amitryptilline	10	6	10-27	16	14	0				n.a.	n.a.	no
Amphetamine	20	2	13-14	14	14	8	30-980	350	307	2.09	0.037	yes
Benzoylec-gonine	50	10	75-581	230	184	13	55-1252	429	408	1.55	0.121	no
Bromazepam	20	15	29-254	98	78	2	108-141	124	124	1.04	0.297	no
Citalopram	5	45	6.7-172	52	48	13	7.8-119	44	28	-0.89	0.376	no
Clonazepam	10	2	11-17	147	14	1	19			n.a.	n.a.	no
Cocaine	10	7	28-690	255	224	8	18-55	37	38	-2.55	0.011	yes
Codeine	10	16	13-277	64	40	2	14-33	23	23	-1.12	0.261	no
Diazepam	20	3	37-99	63	52	8	40-374	176	146	1.63	0.103	no
Ethanol	0.1	217	0.1-2.4	0.5	0.4	156	0.1-4	1.6	1.6	12.29	<0.001	yes
Flunitrazepam	2	0				1	5.9			n.a.	n.a.	no
Lorazepam	10	15	10-83	25	14	12	12-115	39	30	2.00	0.045	yes
MDA	20	0				1	43			n.a.	n.a.	no
MDMA	20	0				2	389-436	413	413	n.a.	n.a.	no
Methadone	10	2	63-350	207	207	5	17-53	42	46	-1.94	0.053	no
Mirtazapine	5	5	10-34	18	16	3	33-38	36	37	1.94	0.053	no
Morphine	10	5	10-104	60	86	5	17-104	45	28	0.10	0.917	no
Nordiazepam	20	23	22-300	85	52	12	28-431	174	148	2.75	0.006	yes
Oxazepam	50	2	144-272	208	208	3	55-86	69	67	-1.73	0.083	no
THC	1	13	1.2-15	5.5	3.7	27	1.2-14	3.5	2.7	-0.95	0.340	no
THCCOOH	5	38	5.1-100	22	18	34	5.7-75	24	16	0.64	0.520	no
Tramadol	50	14	59-723	227	156	2*	464-986	725	725	1.91	0.057	no
Trazodone	10	30	26-296	84	71	7	35-159	68	50	-1.01	0.313	no
Zolpidem	20	13	21-115	48	35	6	32-436	194	185	2.63	0.009	yes

Concentrations are given in ng/mL except for ethanol: g/L

* one outlier of 5098 ng/ml has been disregarded. Although not reported in the hospital record, the high concentration was assumed to be the result of an intravenous tramadol administration at the emergency department before sampling

Table 4.2. Medicinal drugs. Number and percentage of drivers with blood concentrations under, in and above the therapeutic range in the roadside survey (RS) and hospital study (HS).

Substance	Therapeutic range serum-plasma (ng/ml)	Blood/ plasma ratio	Therapeutic range blood (ng/ml)	Under therapeutic range (%)		In therapeutic range (%)		Above therapeutic range (%)	
				RS	HS	RS	HS	RS	HS
Benzodiazepines and Z-drugs									
Alprazolam	5-50	0.8	4-40	0	0	83	0	17	0
Bromazepam	80-170	1*	80-170	53	0	33	100	13	0
Clonazepam	20-70	1*	20-70	100	100	0	0	0	0
Diazepam	100-1500	0.55	55-825	33	12	67	88	0	0
Flunitrazepam	5-15	1.3	6.5-19.5	0	100	0	0	0	0
Lorazepam	20-250	1*	20-250	67	25	33	75	0	0
Nordiazepam	200-800	0.59	118-472	78	25	22	75	0	0
Oxazepam	100-2000	0.9	90-1800	0	100	100	0	0	0
Zolpidem	80-300	1*	80-300	85	33	15	50	0	17
Zopiclone	10-70	1	10-70	0	0	0	0	0	0
Opiates and opioids									
Codeine	Trough 10-50 Peak 50-250	0.87	8.7-43.5 43.5-217.5	0	0	94	100	6	0
Methadone	70-500	0.75	52.5-375	0	80	100	20	0	0
Tramadol			100-800**	21	0	79	33	0	67***
Antidepressants									
Amitriptyline	50-200	0.83	41.5-166	100	0	0	0	0	0
Citalopram	10-200	1*	10-200	7	8	93	92	0	0
Mirtazapine	20-100 (300)	1*	20-100 (300)	80	0	20	100	0	0
Trazodone	300-500 (2500)	0.64	192-320 (1600)	93	100	7	0	0	0

* no ratio available, value was presumed to be 1
** for tramadol the therapeutic range was available for whole blood
*** one case with concentration of 5098 ng/mL not included, see table 1.

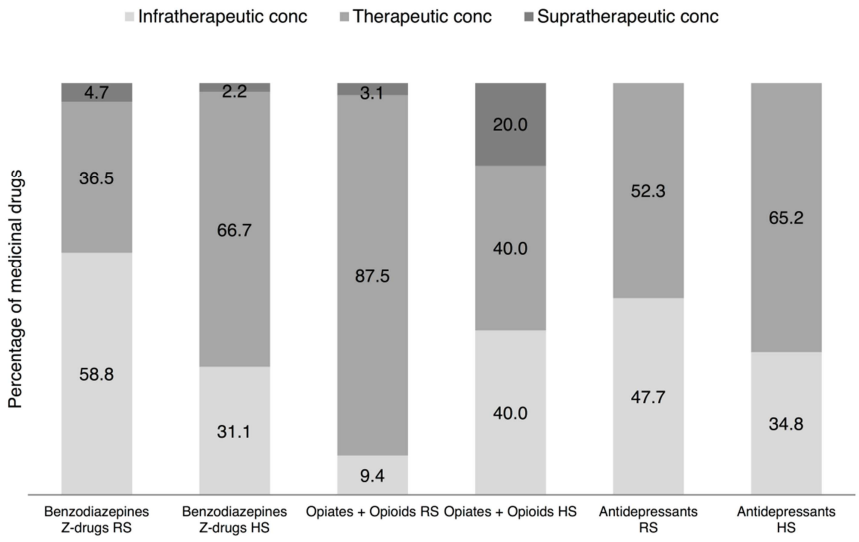

Figure 4.1. Distribution of the concentrations above DRUID cut-off by medicinal drug groups

Discussion

For the interpretation of the data, a series of factors must be taken into consideration.

Concentrations of drugs measured and used in this manuscript were considered separately, however they were not generated from controlled administration of a single drug, and represent the result of different consumption patterns. For example, oxazepam is a medicinal drug itself, but it is also a metabolite of diazepam and nordiazepam. Codeine can be found as a consequence of therapeutic use, but also as metabolite of acetylcodeine, an impurity in street heroin. Also, depending on the time between accident and blood sampling, the concentration of certain drugs may have decreased significantly due to normal metabolism. While during the roadside survey, the blood sample was taken approximately 15 minutes after the driver was stopped, in the injured drivers, the median interval between the accident and the blood sampling was 1.6 hours. This difference in interval means that for the rapidly cleared drugs, especially THC, the blood concentration will probably be lower in the injured drivers due to this added time interval.

Due to the large intra-individual variability in metabolism rate, no attempt was made to 'back-calculate' drug concentrations at the time of the crash.

Although hospital staff was asked to record any drug administered before blood sampling, the possibility that in some cases this procedure was not followed has to be taken into account (e.g. high concentration of tramadol, see Table 4.1).

One would expect higher drug concentrations in drivers injured as a consequence of a crash than in drivers sampled during a roadside survey. This was the case for ethanol, for which the median concentration was 0.4 g/L in the roadside survey versus 1.6 g/L in the injured drivers. Higher concentrations in the injured drivers were also observed for amphetamine, lorazepam, nordiazepam, zolpidem, mirtazapine and tramadol. An opposite trend was observed for cocaine and methadone, with lower concentrations in the injured drivers.

For benzodiazepines and Z-drugs, the concentrations tended to be higher in the injured drivers, while no clear tendency could be observed for the opioids and antidepressants.

Possible drug interactions and pharmacological effect enhancements were also not taken into consideration. Combinations of alcohol and drugs in low concentrations could have synergistic effects. The drug concentration itself is not the only factor determining risk, the differences in the combined use of alcohol and drugs (13.2% in the hospital study vs 0.6% in the roadside survey) and the differences in the median alcohol concentrations (1.6 g/L vs 0.4 g/L) need to be taken into account as well. If a crash is related to the presence of a drug in blood in a dose related manner, it is expected that in injured drivers, the concentrations would be higher than in random drivers. If the drug is used in combination with alcohol, it is expected that (because of the presence of alcohol) lower concentrations of the drug will be enough to impair the driver so he/she is more likely to be involved in an accident. For example in the injured drivers lower amphetamine and cocaine concentrations were found when combined with alcohol. This could suggest that the accident risk is increased either with high amphetamine or cocaine concentrations, or with low concentrations combined with alcohol.

Surprisingly, most of the concentrations of medicinal drugs were in the therapeutic range or lower than the therapeutic range. Further study is needed to determine the role of these infratherapeutic concentrations, if any, in the causation of accidents. Very few medicinal drug concentrations were higher than therapeutic. Supratherapeutic concentrations were found in 0.2% in the randomly selected drivers in the roadside survey and 0.8% in the injured drivers. This indicates that supratherapeutic medicinal drug concentrations are rare in drivers. The fact that no differences in drug concentrations were found for several drugs raises the question whether there is a concentration-effect relationship for crashes for these substances. Although some studies have found indications for drug-concentration related effects on performance, [14,15] very few information can be found on a concentration-effect relationship for accident risk. The low number of non-zero concentrations in the observed populations however, does not allow making too firm conclusions.

For certain drugs, such as Z-drugs, concentrations were mostly in therapeutic range for both groups, but a higher percentage of positive subjects was found in injured drivers. Z-drugs are short acting hypnotics, meant to be eliminated quickly from the body, to avoid next-day effects and therefore, theoretically, to be in subtherapeutic/not active concentrations after a night sleep. Therapeutic concentrations per se may therefore lead to a higher risk of crashes.

Although concentrations of certain drugs were found to be equal in both road side and hospital study, combinations of drugs and alcohol were found in a higher percentage in injured drivers. This might be a possible factor leading to higher accident risk in the latter group.

In general when comparing concentrations distribution in this study with other performed in Luxembourg, [16] Sweden, [17,18] Australia, [19] Switzerland, [20,21] Denmark, [22] the Netherlands[23] and Canada[24] (see Table 4.3), similar or lower results were observed for the Belgian data in both the roadside and hospital study. However it has to be noted that the available data was collected from DUID-cases (drivers suspected to be under impairment), while the present study was based on data from randomly selected drivers voluntarily participating in a roadside survey.

Conclusion

Concentrations of ethanol were significantly higher in the injured population compared to the control group of randomly selected drivers. Higher concentrations in the injured drivers were also observed for amphetamine, lorazepam, nordiazepam and zolpidem. For cocaine, lower concentrations were observed in the injured drivers, although benzoylecgonine concentrations tended to be higher. For cannabis, there was no difference in the distribution of the THC concentrations.

Most medicinal drugs were found in subtherapeutic and therapeutic concentrations, and only 0.2% of the roadside study participants and 0.8% of the injured drivers had supratherapeutic concentrations in their blood. For benzodiazepines and Z-drugs, the concentrations tended to be higher in the injured drivers, while no clear tendency could be observed for the other classes.

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Table 4.3a. Distribution of medicinal drug concentrations in other studies.

Substance	**Concentration range in other studies, Range (median) in ng/mL, [number of positive cases]**								
	Drivers suspected to be under influence/ impaired (DUI)						**(Seriously) injured drivers**		**Killed drivers**
	Appenzeller et al. [16]	Senna et al. [20]	Smink et al [23]	Augsburger et al. [21]	Palmentier et al. [24]	Jones et al [18] *	Drummer et al [19]	Bernhoft et al. [22]	Jones et al. [17]
Alprazolam	20 [1]	6-180 (44) [20]	100-670 (385) [2]			3900 (60) [430]	10-300 (90) [41]	49 [1]	
Amitriptyline	60 [1]	7-350 (85) [6]	57-380 (120) [6]						
Bromazepam	10-1090 [3]	14-1264 (200) [25]	30-1000 (310) [7]					110 [1]	
Citalopram	70 [1]	14-373 (137) [11]			130-200 [2]		10-400 (60) [54]		70-700 (400) [25]
Codeine	4650-7170 [3]	5-290 (11) [125]		1-13 (5) [21]		1000 (10) [617]	10-500 (40) [85]		20-360 (100) [14]
Diazepam	80-980 [6]	8-1140 (118) [52]	10-2500 (277) [166]	80-630 (200) [10]	700-1300 [2]	6200 (200) [1950]	20-7000 (200) [105]	10-60 (40) [6]	60-600 (100) [28]
Lorazepam	20-100 [2]	6-630 (48) [29]	10-85 (50) [12]	10-250 (41) [6]	10-501 [5]				
Methadone			10-930 (151) [88]	27-850 (110) [31]	130 [1]	1100 (200) [114]	30-500 (180) [19]		
Mirtazapine			80 [1]	50-180 (65) [5]			10-400 (40) [17]		100-300 (100) [13]
Morphine	33.9 [1]	5-450 (31) [400]	3-360 (40) [184]	1-111 (10) [32]	15-101 [6]	1600 (30) [864]	10-2900 (50) [30]	50-110 (80) [2]	5-200 (10) [13]
Nordiazepam	60-8810 [18]	6-6500 (270) [95]	9-10300 (260) [197]	30-1560 (305) [24]	100-2200 [3]	5200 (200) [2168]		20-70 (46) [5]	
Oxazepam	10-350 [9]	4-2520 (175) [45]	3-10000 (410) [263]	165-3830 (230) [10]		5700 (800) [49]	20-12000 (40) [19]		
Tramadol	420-1420 [2]			100-950 (425) [4]		7600 (400) [105]	20-1400 (160) [20]		100-6400 (500)
Zolpidem	70-210 [2]	40-1792 (215) [24]	220-2000 (400) [3]	216-600 (340) [5]		3480 (200) [148]			

* only maximum and median concentration are given ** in g/L

Table 4.3b. Distribution of illicit drug and ethanol concentrations in other studies.

Substance	**Concentration range in other studies, Range (median) in ng/mL**								
	Drivers suspected to be under influence/ impaired (DUI)						**(Seriously) injured drivers**		**Killed drivers**
	Appenzeller et al. [16]	Senna et al. [20]	Smink et al. [23]	Augsburger et al. [21]	Palmentier et al. [24]	Jones et al [18] *	Drummer et al. [19]	Bernhoft et al. [22]	Jones et al. [17]
Amphetamine		10-3500 (59) [170]	1-2260 (165) [81]	10-183 (54) [16]			10-60 (30)*** [5]		50-5000 (1100) [39]
Benzoylec-gonine	33.1-642.3 [3]	20-5200 (354) [841]	20-7920 (760) [361]	29-2430 (250) [250]	130-6300 [8]			200 [1]	
Cocaine	1.5-26.9 [4]	10-925 (50) [654]	1-870 (50) [336]	15-560 (50) [20]	130-290 [6]				
Ethanol**	0.2-4.3 [185]	0.15-3.96 (1.21) [1699]	(1.75) [11458]	0.14-2.95 (1.19) [203]			0.1-4.18 (1.07) [498]	0.66-3.10 (1.64) [10]	
MDMA		11-2600 (206) [223]	1-1500 (280) [87]	10-2480 (218) [28]	300-400 [2]		30-300 (50) [13]		
THC	3.1-48.7 [6]	1-62 (3.8) [1704]	0.2-35.3 (4.3) [180]	1-35 (3) [234]	1-10 [18]		2.0-42.0 (7) [167]	1.1-10.3 (2.1) [7]	0.5-9.0 (2) [33]
THCCOOH	5.3-178.4 [20]	1-717 (34) [1619]	0.3-189 (22.5) [257]	1-215 (25) [261]					

** in g/L

*** The low concentrations are explained by the fact that amphetamine is present as the metabolite of methamphetamine. As there were no methamphetamine-positive cases in our study, no concentrations are given.

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CHAPTER 5

Comparison between self-report of cannabis use and toxicological detection of THC/THCCOOH in blood and THC in oral fluid in drivers in a roadside survey

To compare self-report of drug use based on a questionnaire and objective confirmation data, the results of quantitative analyses for cannabis in blood and oral fluid samples were compared to self-reported data on cannabis use. The data of the Belgian roadside study, part of the DRUID WP2, were used for this evaluation.

Self-report data underestimated the use of cannabis and this underestimation was most obvious for recent use.

Personal input:

- Part of data collection
- Toxicological analyses
    - 5-10% oral fluid analyses using UPLC/MSMS for detection of (il)licit drugs
    - 10-15% of alcohol analyses on blood and oral fluid samples using an enzymatic method
    - ELISA and GCMS analyses on blood samples for detection of THC and THCCOOH
- Statistical processing
- Writing the paper

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Abstract

The objective of this study was to compare the number of drivers who self-reported cannabis use by questionnaires to the results of toxicological analysis.

During roadside surveys, 2957 respondents driving a personal car or van completed a questionnaire to report their use of drugs and medicines during the last two weeks and indicate the time of last intake. Cannabis was analysed in oral fluid by UPLC-MS/MS, in blood by GC-MS.

Frequencies in the time categories were calculated and compared with toxicological results. Diagnostic values were calculated for the time categories in which positive findings were to be expected (<4h and <24h for respectively THC and THCCOOH in blood, <12h for THC in oral fluid)

Most self-reported cannabis use was more than 12 hours before driving. The sensitivity of the questionnaire was low, while the specificity and accuracy were high. Kappa statistics revealed a fair agreement between self-report and positive findings for THC in oral fluid and blood and moderate agreement with THCCOOH in blood.

Self-report largely underestimates driving under influence of cannabis, particularly recent cannabis use, therefore analysis of biological samples is necessary.

Introduction

Despite being controlled in many countries, cannabis is the most widely used illicit substance in the world. Results from the 2008 Belgian Health Interview Survey indicated that 14% of the population aged 15-64 years used cannabis at least once in their life, while 5% (one third of them) indicated to have used cannabis in the past 12 months and 3% in the past 30 days. Thirty percent of this last category indicated to smoke it intensively (minimum 20 out of the 30 days). The mean age of first time use of cannabis was 18 years and 11 month.[1]

The prevalence of driving under the influence of drugs such as cannabis has also been studied in recent years. An attitude measurement on traffic safety performed by the Belgian Road Safety Institute (BIVV) in 2009 showed that respectively 13% and 0.76% of the Belgian driving population had declared to have been driving under influence of alcohol or drugs.[2]

Results of the DRUID project have shown that 0.5% of randomly selected drivers in Belgium tested positive for cannabis (0.35% for single use, 0.14% combined with alcohol, medicines or other illicit drugs).[3] The estimated prevalence of cannabis in the general driving population in Europe was 1.32% for single use. [4]

Figures from a study on seriously injured drivers indicated that in Belgium 10% was positive for cannabis (8% in combination with other psychoactive substances, 2% for single use). [5] The prevalence in the five other participating countries (Denmark, Finland, Italy, Lithuania and the Netherlands) ranged from 0.8% to 6.6%.[6]

A case-control study in Belgium estimating accident risk for alcohol, medicines and illegal drugs, demonstrated a concentration-dependent crash risk for THC positive drivers. In general cannabis caused an increase in accident risk with an odds ratio of 13.4. [7]

Risk analysis based on the overall DRUID case-control data showed that the risk associated with cannabis seems to be similar to the risk when driving with a low alcohol concentration (between 0.1 and 0.5 g/L), which is about 1-3 times that of sober drivers. [8]

Meta-analyses performed by Asbridge, [9] Li et al. [10] and Elvik[11] also suggest that cannabis use by drivers is associated with a significantly increased risk of being involved in motor vehicle crashes.

Much of the early research assessing the effects of cannabis on driving performance was done by laboratory and driving simulator studies. The results of these studies are generally consistent: at increased doses, cannabis impairs the psychomotor skills necessary for safe driving. [12–19]

Fergusson and Horwood[20] found that the risk of crash involvement increased significantly as self-reported frequency of cannabis use in the past year increased.

Evaluation of drug use based on the subject's self-report is the most widely used practice for epidemiological research in addiction, as it has two very clear advantages, low cost and the possibility

of collecting an abundance of information from many people. [21] However, the validity of estimations based on their use has frequently been questioned. There has been a certain tendency to believe that results from self-reported use are only the 'tip of the iceberg' of real consumption and that therefore, the studies estimating the highest prevalence were the most valid, although this affirmation has also been questioned. [22]

Drug use is frequently considered within a social-cultural framework as improper, shameful, dangerous and even illegal, so that the subject's own report on it may be subject to deception, hiding and other types of bias in the response. [23]

The purpose of this study was to compare the number of drivers who declared to have used cannabis with the results of toxicological analysis of an oral fluid sample and blood sample. Is the self-report of cannabis use by drivers biased? To what extent is there a correlation between both types of information on use (self-report versus toxicological analyses)?

Method

Participants

Between 2008 and 2009, 2957 respondents driving a personal car or van participated in a roadside survey.

Sixty-seven percent (1989) of the drivers were male and 32.7% (967) female. Almost 58% of the drivers could be categorised in the age group 25-34 (20.7%) or in the group 35-49 (37.2%). The percentage of respondents in the categories 25-34 and 50+ were 11.4 and 30.0 respectively.

Variables and instruments

Two techniques were used a) self-report, based on a self-administered questionnaire, and b) analysis of blood and oral fluid samples to measure use of cannabis.

a) Self-report

A questionnaire was given to the respondents of the roadside survey to report their use of drugs and medicines during the last two weeks and indicate the time of last intake.

Following data were recorded: type of vehicle; gender; age; education level; result of breathalyser test, drug control or other observations by police and self-reported drug, alcohol and medicine use.

The respondents were asked to fill in their questionnaire, while waiting for the oral fluid sample to be collected. Questions could be asked to the research staff when topics were not clear. No interviewing was done. The surveys were guided by a member of the research team or a trained student. The interviewers went quickly through the completed questionnaires and if important information was not completed, this was specifically requested at the respondent.

b) Toxicological analysis

Each volunteer was asked to provide a blood sample (5mL tube with sodium fluoride and potassium oxalate) and an oral fluid sample collected with the StatSure™ Saliva Sampler ™. The collection device consists of a cellulose pad on a plastic stick. When approximately 1ml sample has been collected, an indicator on the stick turns blue. The stick was then sealed in a tube containing 1ml of buffer. The oral fluid samples were weighted to correct differences in sample volume. [24]

A total of 2750 drivers provided both a blood and an oral fluid sample, while 199 drivers only provided an oral fluid sample and 8 drivers only completed a questionnaire.

Samples were transported under cooled conditions to the laboratory where the toxicological analyses for 11 illicit psychoactive substances and metabolites[1] were performed.

In oral fluid THC was analysed using liquid-liquid extraction (LLE) followed by UPLC-MS/MS. [25] Blood samples were initially screened for using ELISA and confirmed using LLE followed by GC/MS. [26] All the blood samples for which the corresponding oral fluid sample was positive for THC as well as 300 for which the corresponding oral fluid sample was negative for THC were screened with ELISA (IDS Elisa One-Step Cannabis (Cat No. TH-96-CE-U), targeting THCCOOH)[2].

The cut-off for THC was set at 1 ng/mL for both matrices. For THCCOOH in blood, a cut-off of 5 ng/mL was used.

Survey Procedure

The research procedure consisted of two independent phases: the first was an random alcohol control performed by the police. After the police procedure the stopped drivers were asked whether they wanted to participate in the DRUID-research. If they refused, a refusal form with demographic data of the persons was filled in to be able to calculate a response rate. The second phase was the DRUID research itself, which took place in a motorhome. The drivers were informed about the objective and the content of the research, asked to fill in a questionnaire, to give an oral fluid sample and a blood sample. Drivers who didn't want to participate in the study where asked to only fill in the questionnaire. If they refused a refusal form was filled in to be able to calculate a response rate. Respondents who participated in the study were given compensation in form of a gift voucher of € 20. Survey sessions lasted 90 minutes at one location. [3]

The project was conducted according to the guidelines laid down in the Declaration of Helsinki and was approved by the Ethics Committee of Ghent University Hospital. (Belgian registration number B67020073143).

[1] 6-acetylmorphine, amphetamine, benzoylecgonine, cocaine, MDA, MDEA, MDMA, methamphetamine, morphine, THC and THCCOOH.

[2] Concentration (ng/mL) that gives a positive response (equivalent to xx ng/mL of THCCOOH): 11-nor-delta-9THC-9-COOH=4100; Delta-9-THC= 7; Delta-8-THC=5; 11-nor-delta-8-THC-9COOH= 87.5; 11-nor-delta-9-THC-9-COOH-glucuronide=50; 11-Hydroxy-delta-9-THC= 21.9; Cannabinol= 2.7; Cannabidiol=0.002

The respondents were assured confidentiality. Anonymity was guaranteed by linking toxicological and questionnaire data through numbers.

Data analysis

Percentages of positive findings and concentration ranges were calculated using Microsoft Office Excel 2010. Statistical analysis was made using IBM SPSS Statistics 21.

Following data were calculated: ratio of positive toxicological result / self-report use by category of 'time of intake'; diagnostic values (sensitivity, specificity, accuracy) and kappa statistics.

The evaluation of the results is based on classification into the following categories

True positive (TP): number of cases with a positive self-report and a positive confirmation analysis

True negative (TN): number of cases with a negative self-report and a negative confirmation analysis

False positive (FP): number of cases with a positive self-report and a negative confirmation analysis

False negative (FN): number of cases with a negative self-report and a positive confirmation analysis

Since we expect, according our cut-off and the Model 1 formula of Huestis,[27] THC to be positive in blood for up to 5 hours after intake, positive self-report was defined as those respondents who declared to have use cannabis 'less than 4 hours ago'.

For THCCOOH in blood and THC in oral fluid these limits are set to '< 24 hours' and '<12 hours' respectively.

Using these classifications, the following parameters for the evaluation can be calculated:

Sensitivity is the proportion of positive cases (= subjects with THC/THCCOOH in blood or oral fluid) that are correctly identified by the test (= self-report of cannabis use).

$$Sensitivity = \frac{TP}{TP + FN}$$

Specificity is the proportion of negative cases (= subjects with no THC/THCOOH in blood or oral fluid) that are correctly identified by the test (= self-report of no cannabis use).

$$Specificity = \frac{TN}{TN + FP}$$

Accuracy is the proportion of correctly identified positive and negative results from all the test results.

$$Accuracy = \frac{TP + TN}{TP + TN + FP + FN}$$

Sensitivity, specificity and accuracy performance values of 80% or more were set as a desirable target value.
Cohen's kappa measures the agreement between two raters who each classify *N* items into *C* mutually exclusive categories. The equation for κ is:

$$K = \frac{\Pr(a) - \Pr(e)}{1 - \Pr(e)}$$

Where Pr(*a*) is the relative observed agreement among raters, and Pr(*e*) is the hypothetical probability of chance agreement, using the observed data to calculate the probabilities of each observer randomly saying each category. If the raters are in complete agreement then κ = 1. If there is no agreement among the raters other than what would be expected by chance (as defined by Pr(*e*)), κ = 0.

Interpretation of Kappa is rather arbitrary. Landis and Koch characterised values < 0 as indicating no agreement and 0–0.20 as slight, 0.21–0.40 as fair, 0.41–0.60 as moderate, 0.61–0.80 as substantial, and 0.81–1 as almost perfect agreement. [28] Fleiss's equally arbitrary guidelines characterize kappa over 0.75 as excellent, 0.40 to 0.75 as fair to good, and below 0.40 as poor. [29]

Three box and whisker plots were drawn (one for each analyte), to show the distribution of the non-zero concentrations by self-reported time after intake. The box in these box and whisker plots represents those cases between the 75th and 25th percentile (Q3-Q1), whilst the line that bisects the box is the median concentration of the cases. The whiskers that protrude from the box extend to 1.5 times „Q3-Q1„ or, if no case has a value in that range, to the minimum or maximum values. If the data are distributed normally, approximately 95% of the cases are expected to lie between the whiskers. Outliers, denoted by a point, are defined as cases that do not fall within the whiskers. Extreme outliers are denoted by asterisks and represent cases that have values more than three times „Q3-Q1‟ beyond the limits of the box.

Results

Table 5.1 shows the relationship between the time after intake and the ratio of positive toxicological result versus self-reported use.

Out of the 81 persons who declared to have used cannabis, 34 were found positive for THC in oral fluid. The ratio of positive toxicological result versus self-reported use was highest at ‘<1h’ and ‘<12h’, with a decline after 12 hours.

For blood results, only 8 out of 81 self-reported users were found positive for THC, 27 were positive for THCCOOH. The ratio of positive toxicological result versus self-reported use for THCCOOH is the highest at '<1h' (0.8), showing a decreasing trend with increasing time after intake. The ratio for THC is indicating a peak at 4 hours, a possible outlier at 12h and the same decreasing trend at 24h and more after intake as THCCOOH in blood and THC in oral fluid.

For 5 self-reported users, only oral fluid results were available, since either no blood sample was taken, or not enough sample was left for THC and THCCOOH analysis. They were only included in the OF analysis.

Table 5.1. Self-reported cannabis use and positive toxicological results per time category of intake

	Total	<1h	<4h	<12h	<24h	>24h	unknown
Total number of subjects who self-reported cannabis use	81	5	3	10	7	46	10
Number of positives in saliva for THC (cut-off: 1 ng/mL) among the subjects who self-reported cannabis use	34	4	1	8	3	15	3
Number of positives in blood for THC (cut-off: 1 ng/mL) among the subjects who self-reported cannabis use	8	1	2	0	1	4	0
Number of positives in blood for THCCOOH (cut-off: 5 ng/mL) among the subjects who self-reported cannabis use	27	4	2	5	3	11	2
Number of subjects who self-reported cannabis use while no THC or THCCOOH were detected in blood or oral fluid	43	1	1	2	3	29	7

Figure 5.1 gives an overview of the distribution of THC concentrations in oral fluid and THC and THCCOOH concentrations in blood. It shows that, as generally known, THC concentrations are higher in oral fluid then in blood. Generally speaking higher THC concentrations are found in oral fluid for the time categories '<1h' and '<4h', a decline after 12 h is noticeable and stable for the following categories. THCCOOH concentrations are quite equal for all time categories with a small decrease in the category '<12h'. For THC in blood, the low number of positive findings (n=8) might give a biased idea. The concentrations seem to be increasing until '<24h' and then rapidly declining.

The cases with negative self-report but positive toxicological results were investigated using the formulas of Huestis [27] to estimate the time of intake. Out of 48 cases in total, only four datasets were complete with both THC and THCCOOH results. The calculated time ranged between 0.7 and 2.1 hours (CI: 0.3-4.7 hours) according to model 1 and between 1.3 and 2.6 hours according to model 2 (CI: 0.5-7.0 hours).

Table 5.2 gives an overview of the diagnostic values and the kappa statistics calculated for the self-report versus toxicological analysis, with the toxicological results considered as the reference method.

Sensitivity is low for all three analytes, only higher than 50 % for THCCOOH in blood. Specificity is ranging from 94 for THC in blood to almost 100% for THC in oral fluid.

Kappa statistics can be read as fair for THC in oral fluid and in blood and moderate for THCCOOH in blood.

Of the 47 false positives in oral fluid (positive self-report but negative oral fluid results), one respondent who declared to have smoked cannabis less than 4 hours ago, tested positive for THC and THCCOOH in blood. One person categorised in '<24h' and two in '>24h' were positive for THCCOOH in blood.
Of the 33 false positives for THC in blood (positive self-report but negative for THC in blood), almost 70% had concentrations of THC in oral fluid and/or THCCOOH in blood above the cut-off. The same trend could be seen for the false positives for THCCOOH.

Table 5.2. Diagnostic values and kappa statistics for self-reported cannabis use (<4h for THC in blood, <12h for THC in oral fluid and <24h for THCCOOH in blood) by laboratory test.

	THC oral fluid (cut-off: 1 ng/mL)	**THC blood (cut-off: 1ng/mL)**	**THCCOOH blood (cut-off: 5 ng/mL)**
TP	13	3	14
TN	2829	64	57
FP	5	4	3
FN	47	5	11
Total	2894	76	85
Sensitivity	0.22	0.38	0.58
Specificity	0.998	0.94	0.95
Accuracy	0.98	0.88	0.84
Kappa	0.33	0.34	0.56

TP=positive self-report + positive toxicological analysis
TN=negative self-report + negative toxicological analysis
FP=positive self-report + negative toxicological analysis
FN=negative self-report + positive toxicological analysis
Sensitivity: proportion of subjects in whom THC/THCCOOH was detected in OF/blood that self-reported cannabis use
Specificity: proportion of subjects in whom no THC/THCCOOH was detected in OF/blood, that self-reported no cannabis use
Accuracy: proportion of subjects who accurately self-reported cannabis use

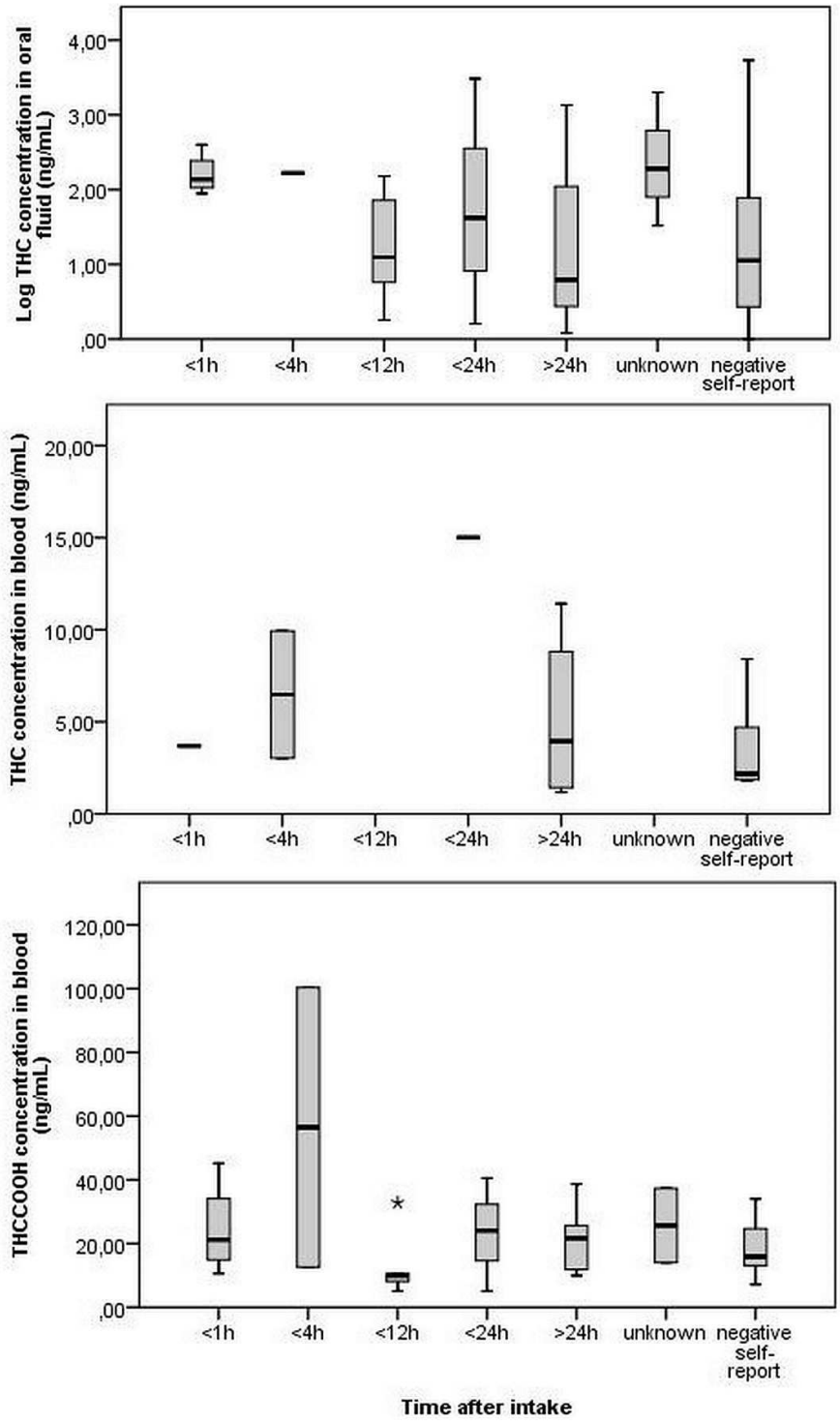

Figure 5.1. Distribution of analyte concentrations by time after intake

Discussion

Concentrations of THC depend on dose and type of use (occasional or chronic). Also, after cannabis inhalation, contamination of THC in the oral cavity appears. These facts may explain the rather irregular pattern of the ratio between self-reported use and toxicological findings in the first hours, and a declining trend after 12 hours, as expected with plasma concentrations.

A study in 2003 compared self-report data and oral fluid testing in patients treated for drug addiction. Findings indicated a high level of consistency between self-reported drug use and oral fluid testing. However, agreement varied by drug type and respondents commonly reported consumption that screening failed to identify. Inconsistencies appeared to relate to a number of factors and were not necessarily a function of deliberate distortion by the drug user. [30] This study was conducted on new treatment patients, which is a different population compared to randomly selected drivers.

A study in 2009 compared self-report of cannabis use by university students with detection in urine. Sensitivity of the self-report was 91.8%, the specificity was 89.6%.[31]

One of the key elements of a questionnaire, is the way of interviewing: orally or with a written questionnaire. Who is responsible for the interview: an expert, a student,…? In our study interrogation was not performed by expert interviewers and there was little interaction between respondents and the study team. Future research might benefit having well-trained interviewers who work in drug advisory clinics. Getting into dialogue with the respondents could reduce the number of incomplete questionnaires. But it still has to be kept in mind that a roadside setting is different from drug advisory clinics where fear of retribution is less and cooperation is part of the treatment.

Also the illegal nature of drug abuse, privacy, face-saving and possible criminal sanctions are all factors associated with social pressure, largely affecting the reliability of self-report outcomes. However, in Belgium cannabis possession of less than 3 grams for one's own use is not prosecuted, so users might be more willing to declare use than in other countries.

Although it was explained to the respondents of the roadside survey that there was no data transmission from the study to the police forces, there could have been a bias: people were maybe more reluctant to share information with police forces nearby. Since a consent form had to be signed, some respondents could not reconcile this with the guaranteed anonymity. Also some participants asked to be updated on the test results, indicating that the term anonymity was not always fully understood.

The preceding police procedure might have induced restlessness in some respondents, which did not attenuate completely once they were completing the questionnaire, even though confidentiality was observed. Those participants might have volunteered more out of fear than for altruistic reasons or for a reward. This could explain the conclusion we made out of our data, that respondents gave a more 'socially desirable' answer, e.g. reporting use more than 24 hours ago, while in fact biological analysis suggest use within a period of 4 hours. It has to be taken into account that since 7 (5 gave an oral

fluid sample, 2 only filled in the questionnaire) of the self-reported users did not provide a blood sample, and the already low number of positives in blood, making assumptions based on blood results should be done carefully. Especially because Van der Linden et al. [24] demonstrated that there was a higher percentage of drug-positive drivers in a group of respondents who did not provide a blood sample compared to the group who give a blood and oral fluid sample.

The following remarks could be made regarding the false positive results. The person with self-reported use less than one hour before, with negative results for THC in oral fluid, but THC and THCCOOH in blood, might be missed as positive in oral fluid due to a sampling problem. Of all negative saliva samples whose corresponding blood was analysed (300 in total), this respondent was the only one found positive in blood. The calculated time after intake with the formulas of Huestis [27] suggest recent intake.
The three positive self-reports with negative result for THC in blood, categorised in '<1h' were positive for THC in oral fluid and THCCOOH in blood. This could suggest chronic use (indicated by the residual THCCOOH in blood), with very recent last intake.
The positive self-reports with negative result for THCCOOH might suggest more occasional use, since there was no residual THCCOOH in blood.

Data on the 5 false negatives (no self-reported use, but positive toxicological results) for whom with blood concentrations and the formulas of Huestis [27] time of intake was estimated, suggest that these respondents had smoked very recently and hence probably didn't give a correct answer to the questionnaire.

Subjects were asked to report their use within the past 14 days, subdivided into several categories (<1h, <4h, <12h, <24h, >24h, unknown). It has to be noted that '>24h' was a widely defined category.

Looking at the distribution of the concentrations it could be noted that maybe some respondents gave a 'socially desirable' answer, stating 'I smoked cannabis >24 h ago', while in fact it was more recently.

Kappa Statistics are fair to moderate and specificity is very high. But since sensitivity and prevalence of use in the general driving population (0.5%) [3] are low, positive predictive values (PPV)[3] calculated through the theorem of Bayes, in which PPV is directly proportional to the prevalence, are low. For instance, using the values for sensitivity and specificity for THC in blood, a prevalence of 38% is needed to have a positive predictive value of 80%.

Future roadside studies based solely on self-report might not be worthwhile as the prevalence of recent drug use is severely underestimated. Objective measurements on biological samples give more accurate information. On the other hand, such questionnaire data could be used to gather extra information (like time of last use and route of administration) for traffic statistics.

[3] PPV= $(sensitivity * prevalence)/(sensitivity * prevalence + (1 - specificity)(1 - prevalence))$

Conclusion

Although in other settings the use of self-report turned out to be a good indicator for cannabis use, the presented data suggest that self-report is not the ideal measurement to detect driving under influence of cannabis. The idea of a possible retribution or penalty associated with a positive answer might have a great impact on the way of completing the questionnaire. Analysis of biological samples is more accurate.

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CHAPTER 6

EXTRA RESEARCH

Some unpublished data that highlights the role of quantitative bio-analysis in epidemiological studies of drugs and driving is given.

Chapter 6 describes some new data and data that were not included in the publications.

1. Prediction of time of cannabis use from plasma concentrations of THC and THCCOOH in a roadside survey and a seriously injured drivers study.

The time of last cannabis use for roadside respondents and seriously injured drivers [1] was estimated with positive plasma results for THC and its metabolite THCCOOH by using the models of Huestis et al. [2] Only drivers for which plasma THC levels were greater than or equal to 2.0 ng/mL were included according to the exclusion criteria of Huestis et al.

Model 1 only looks at the THC concentration, while model 2 also incorporates THCCOOH concentration (see Table 6.1), the latter being less accurate for frequent users since regular smokers often have residual concentrations due to prior administration.

Table 6.1. Models to predict cannabis exposure [2]

	Model 1	Model 2
$Log\ T\ (h)$	$-0.698 * log\ [THC] + 0.687$	$(0.576 * log\ [THCCOOH/THC]) - 0.176$
$CI = Log\ T \pm 1.975$	$\sqrt{0.030(1.006 + \{(\log[THC] - 0.996)^2/89.937\})}$	$\sqrt{0.045(1.006 + \{(\log[THCCOOH]/[THC] - 0.283)^2/123.420\})}$

In the roadside survey 13 subjects had a THC plasma level higher than or equal to 2 ng/mL. In the injured drivers study this number was 56.

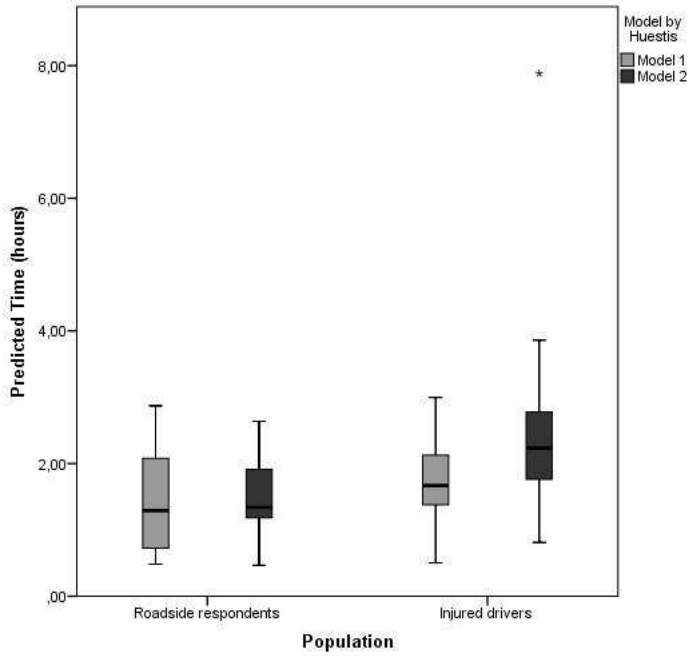

Figure 6.1. Boxplot of predicted time of use in roadside and injured drivers study according to Model 1 and 2 of Huestis.

Using model 1 of Huestis et al., the predicted time of last cannabis use varied from 0.5h to almost 3h. Using model 2, the range stayed the same for the roadside survey, but for the injured drivers the range became wider from 0.8h to almost 8h (see Figure 6.1).

In each single case, the confidence intervals of both model 1 and model 2 overlapped, suggesting similar predictions.

As already stated by Huestis et al., [2] model 2 seemed less accurate for frequent users since regular smokers often have residual concentrations due to prior administration. In that respect, it seems that the results from model 1 might be more accurate since they are more reliable in frequent users, However, data on the frequency of use were not available in our study. In addition, blood sampling was performed with a delay of maximum 3 hours after the accident. In this time frame, the blood concentration of THC drops considerably, which makes the use of model 2, which also incorporates the THCCOOH concentration, a better option.

Using the models of Huestis, the predicted time of use in the roadside and injured drivers study indicated recent intake of cannabis before getting behind the wheel.

2. Using roadside data to predict prevalence in general population?

We also studied whether roadside data containing quantitative drug analysis results on psychoactive substances can be used to predict prevalence of drug use in the general population, for which self-reported data is the only source of information.

Gjerde et al. [3] investigated the possibility of using roadside prevalence as representative prevalence for the whole population, concluding this could be the case for THC. Based on this article, additional research was performed to see whether data on cannabis use in the Belgian roadside survey could be used to estimate use in the general population. The results of the Belgian roadside survey on drugs and driving [4,5] using oral fluid analysis and questionnaires to estimate the use of cannabis were compared with the data on self-reported use of cannabis from the Health Interview Survey (HIS) in Belgium. [6] Through a webpage for interactive analysis (https://www.wiv-isp.be/epidemio/hisia/) prevalence of use of drugs and medicines was calculated. Comparable data were available for last month prevalence of use of cannabis. This prevalence was calculated for the age group 17-86 year, being the age-range of the roadside study. The results presented are weighted percentages and their 95% confidence intervals (CI), together with the total number of respondents (N is unweighted). The use of weighting factors adjusted for differences between the survey sample and the real population, in terms of the distribution by age, sex, size of the household and province. By weighting the data the results were representative for the total population, at national, regional and provincial level. [6]

For the roadside data, 2957 respondents driving a personal car or van completed a questionnaire during roadside surveys to report their use of drugs and medicines during the last two weeks and indicated the time of last intake. Besides questionnaire data, oral fluid samples were collected and

analysed with UPLC-MSMS with a cut-off of 1 ng/mL for THC. [4,5] Data of the roadside survey (toxicological and based on questionnaire) were weighted for time period. [5,7]

Table 6.24. Percentage of respondents who have indicated that they had used cannabis.

HIS data (confidence interval) on use in the last month	Self-reported use in last two weeks in roadside survey	Positive oral fluid sample in roadside survey
3.1 (2.4-3.8) (data 2008) 2.6 (2.0-3.2) (data 2013)	2.3	2.4

In the general population survey, 3.1% and 2.6% (respectively in 2008 and 2013) declared to have used cannabis in the last month. In the roadside study, only the prevalence of last 2 weeks use was asked for, being 2.3%. Of these, 40.7% tested positive for THC in oral fluid. The prevalence of positive oral fluid samples within the total group of respondents was 2.3, meaning that for a number of drivers the self-report was negative and the oral fluid result positive. The difference in period (month versus 2 weeks) limits the comparison but because the roadside data were situated in the confidence interval of the HIS, a similar prevalence in both groups could be assumed (see Table 6.2).

Although these data seem promising, self-report in the general population is still necessary to gather more information like time of last use and route of administration. Roadside data are for instance not suitable to assess the prevalence of last year use; this was clear from the comparison of the European estimate of last year cannabis use by adults in 2010 (6.8%) [8] and the European mean value of the DRUID roadside study (1.3-1.7%). [7] Even with the estimate of last month use (3.6%), [8] a larger difference is reported compared to the Belgian data.

3. Self-report of other psychoactive substances

Comparisons between self-report and toxicological analysis for cannabis and other psychoactive substances were presented at the 2010 TIAFT congress. [9]

During roadside surveys, respondents driving a personal car or van completed a questionnaire to report their use of drugs and medicines during the last two weeks and indicate the time of last intake. Frequencies in the time categories were calculated and compared with toxicological results. Psychoactive substances were analysed in oral fluid by UPLC-MSMS. [4,7]

Figure 6.2 shows the relationship between the ratio of positive toxicological result vs self-reported use and the reported time after intake for the most frequently detected psychoactive substances: codeine, THC, benzodiazepines and Z-drugs, antidepressants and alcohol.

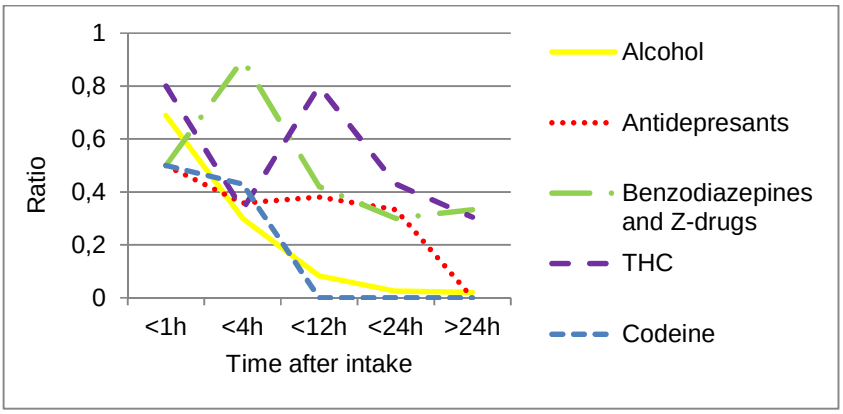

Figure 6.2. ratio positive toxicological result/self-reported use versus time after intake

According to self-report, most drugs were last taken 4h or more before driving. Self-report yielded more positives than toxicological analysis.

For alcohol, the ratio of self-reported use versus toxicological findings decreased over time, reflecting the normal kinetics of alcohol elimination.

For antidepressants the ratio appeared to be more constant over the 24 hours. This pattern is in good agreement with a long-term therapy and longer half-lives, aiming to achieve a steady state concentration. It has to be noted that toxicological analysis did not screen for all the available antidepressants, which might explain why the low ratio between toxicological findings and self-report.

Since for benzodiazepines and Z-drugs, peak plasma concentrations of the target substances were situated between 1 and 4 hours after intake, this could explain the ratio peaking at <4h. The apparent stable ratio after 12h is probably due to the wide range of half-life for the analysed drugs.

Concentrations of THC depend on the dose and the type of use (occasional or chronic). After cannabis inhalation, contamination of THC in the oral cavity does occur. These facts may explain the rather irregular pattern of the ratio between toxicological findings and self-reported use in the first hours, and a declining trend after 12 hours, as expected with declining plasma concentrations.

For codeine, the pattern that emerged is likely to suggest occasional use, maybe at low dose in combination with acetaminophen (paracetamol), as it is with the consumption of cold medicines.

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CHAPTER 7

GENERAL DISCUSSION

The role of quantitative bio-analysis in epidemiological studies of drugs and driving is highlighted.

Chapter 7 summarizes the main results of this dissertation and gives recommendations for possible future research.

In this dissertation we evaluated the use of bioanalysis in studies on drugs and driving in different driver populations, with special attention to the value of quantitative analysis. The effect of using similar (in order of magnitude) cut-off values in blood and oral fluid was investigated as was the effect of the implementation of the new Belgian legislation on the number of false positive screening results. The distribution of the concentrations of several psychoactive substances was compared between the general driving population and seriously injured drivers. The validity of self-reporting of cannabis use was determined based on quantitative analysis on oral fluid and blood.

One could argue that, especially for routine DUID testing, reporting quantitative results is not necessary, especially when the approach is actually a zero tolerance one. However, the use of validated quantitative methods is a prerequisite to apply standardised cut-off values whether they are analytical or based on impairment levels.

Since the correlation between drug concentrations in blood or oral fluid and the level of impairment remains unclear, some countries have introduced *per se* laws with a zero tolerance character. Some (particularly with a low BAC limit of 0.2 g/L) have chosen this approach since it is in line with the approach for driving under the influence of alcohol. Quantitative bioanalysis is useful to collect data on the actual concentrations found in the biological fluids, and to detect concentrations below the cut-off level in order to, if necessary, lower cut-offs when for instance new data on levels of impairment become available. Such a *per se* legislation based on risk thresholds is implemented for driving under influence of alcohol, where 0.5 g/L was set as a 'danger' cut-off. This was based on thorough quantitative research showing a doubling of accident risk at a blood alcohol concentration of 0.5 g/L.

Whereas a zero-tolerance legislation (with analytical cut-offs) might be used when targeting driving under influence of illicit drugs, it might be better to approach the problem of driving under influence of psychoactive medicines differently. Although medicines cannot be categorised under the same term as illicit drugs, some medicinal substances are equally impairing. Some people need medicines and need to be mobile to have a fulfilling social life. On the other hand, it cannot be allowed that people drive when they are putting themselves and others at risk because their driving abilities are impaired by medicines. In case of the use of legal (medicinal) drugs in traffic, policy makers have to find a balance between traffic safety issues for all and the therapeutic needs of the single road user. Many drivers also need medicinal substances to restore their fitness to drive. Consequently, it is not necessary to deter them but to increase preventive efforts, in particular educative measures. For medicinal drugs, a law where cut-offs are set to indicate the level of impairment might provide a solution. The main disadvantage of this type of law is the complicated enforcement procedure. [1] Another disadvantage of impairment cut-offs is the fact that they do not take tolerance into account: for certain medicines the risk of accident involvement is high at the start of treatment, but after a certain period of time tolerance to the impairing effect is observed, resulting in a decrease of risk.

This chapter starts with a general discussion based on the published followed by the impact of enforcement and future perspectives regarding new psychoactive substances, biological matrices and further research.

1. Discussion on chapters 2 to 6

1.1. DUID: oral fluid and blood confirmation compared in Belgium

Chapter 2 described the results of the confirmation analyses of paired samples of blood and oral fluid from 2949 respondents out of a group of randomly selected drivers. The aim of the study was to investigate the percentage of drivers who test positive in each confirmation matrix. [2]

A remarkable observation was the higher number of positives in a group that only provided an oral fluid sample compared to the group for which both blood and oral fluid samples were available. This could indicate some fear of being found positive in blood and therefore being reluctant to provide this biological fluid for analysis, even in the context of an anonymous study.

Because most components are present in higher concentrations in oral fluid than in blood, more persons are expected to test positive when oral fluid is used as matrix when similar (or even lower cut-offs) than in blood are used. This was also reported in the general DRUID-study. When using the original analytical DRUID cut-offs, [3] for several substances a difference in number of positives could be observed in oral fluid than in blood. To approach this disparity, equivalent cut-offs were calculated and used for the final calculation of prevalence. [4] These equivalent oral fluid cut-offs were compared to the legal Belgian cut-offs for the dataset used in Chapter 2. [2]

This resulted in comparable numbers of positive respondents when using blood or oral fluid for cocaine and benzoylecgonine. For THC, the prevalence when using the oral fluid data was still higher than using the blood data but to a lesser extent (1.1%, versus 0.47% in blood, see Table 7.1).

Table 7.1. Data on drivers who tested positive in the Belgian roadside survey according to legal and equivalent cut-offs (ng/mL)

Substance	DRUID cut-offs in blood (ng/mL)	DRUID equivalent cut-offs in oral fluid (ng/mL)	Positive according to cut-offs N (%)			Statistics	
			OF Belgium	Blood Belgium	OF equivalent	Chi²	p-value
THC	1	27	40 (1.45)	13 (0.47)	30 (1.1)	A: 12.88 B: 6.00	A: <0.001 B: 0.0143
Amphetamine	20	360	2 (0.07)	0	0		
MDMA	20	270	0	0	0		
Morphine	10	95	12 (0.44)	5 (0.18)	4 (0.15)	A: 2.12 B: Fisher	A: 0.1454 B: 1.000
6-monoacetylmorphine	10	16	3 (0.11)	2 (0.07)	3 (0.11)	Fisher	1.000
Cocaine	10	170	25 (0.91)	7 (0.25)	11 (0.40)	A: 9.08 B: 0.50	A: 0.0026 B: 0.4795
Benzoylecgonine	50	95	25 (0.91)	12 (0.44)	12 (0.44)	A: 3.92 B: 0.04	A: 0.0477 B: 0.8415

A: comparison between blood and oral fluid with the cut-offs of the Belgian law, [2]

B: comparison between blood and oral fluid using equivalent OF cut-offs. [4]

By using similar confirmation cut-offs in blood and oral fluid, or even lower ones in the latter, differences in the number of positive results were created. Investigating the quantitative analyses of paired samples has shown that more drivers were prosecuted when analysis was performed in oral fluid than when confirmation results in blood were used. This might be a disadvantage to study drug prevalence in the general driving population but in the context of zero-tolerance, a step forward in combatting DUID. It was also necessary to set the cut-offs sufficiently low to minimise the percentage of false positive screenings, taking into account the low screening cut-offs of the onsite tests.

1.2. Road side drug testing: Comparison of two legal approaches in Belgium

In chapter 3 we compared the number of true and false positives between two legal approaches with a different roadside procedure for detection of drugged drivers, different screening methods and different confirmation cut-offs in a large data set from consecutive time periods. The objectives of the study were to investigate if the total number of false positive screenings had dropped since the new legislation was applied, to see if this expected decrease was confirmed for all substance classes and to investigate which change in the legislation had led to the increase of true positives: the change of screening method or the lowering of the confirmation cut-offs.

Quantitative analysis has shown that with the new approach using on-site oral fluid tests and lower confirmation plasma cut-offs, fewer false positive screening results were observed and recent drug use was targeted, especially for THC. [5]

The data of the publication in chapter 3 were collected from April 1st 2008 to March 31st 2013. From mid-2013 onwards, an adapted version of the oral fluid on-site test was used, with adjustments to the immunological assays for THC and cocaine/benzoylecgonine. An extra set of data collected during 2014 (n= 2698), with the application of the adapted screening device, was studied. The percentage of plasma samples of tested drivers, in which none of the positive screened target drugs were present in a concentration above the legal cut-off value further decreased to 7%. For THC, the percentage of false positives decreased further to 6%, probably because of the improved readability of the test. The median plasma cocaine concentration decreased from 47 to 34 ng/mL using respectively the old version and the new oral fluid device ($p<0.001$). More plasma samples contained only benzoylecgonine (without cocaine) but no significant difference was observed ($p=0.073$). [6]
Our results also suggest that commonly used opioid-like medication did not contribute to a large extent to the high number of false positive screening results for opiates. Further research on the prevalence of different ATS with regards to the results of the amphetamine screening test would be useful. In addition, the role of new psychoactive substances should be investigated, even if currently a low NPS use is observed in Belgium. [7,8]

When comparing two (or more) datasets, it is also important to look for possible confounders, to check if the observed changes are not biased by other items that could give rise to changes in the threshold or the motives to collect samples. Possible confounders are a shortage in onsite tests or

defect batches. Since the number of performed tests (per dataset) was constant throughout the years, of this study, this does not seem to have an effect. Another confounder could be the shortage of a certain illicit drug on the market within a certain period. This was for instance the case for MDMA which had almost disappeared from the Belgium illicit drug market in the period 2008-2009 (period of urine screening), probably due to a shortage in the supply of the precursor. Since 2010 (period of oral fluid screening) MDMA regained its place on the Belgian market, suggesting this could be a confounder for the small difference in percentage of MDMA confirmation in plasma between both screening periods.

1.3. Comparison of drug concentrations measured in roadside surveys and in seriously injured drivers

In chapter 4 we compared the concentrations of psychoactive substances between the general driving population and injured drivers, to investigate whether higher concentrations would be detected in the blood samples of injured drivers.

Calculating risk estimates can be done solely by comparing cases and controls based on their categorisation in positive/negative for certain substances. However, using quantitative results provides more detailed information. For instance, the percentage of ***alcohol*** positives was not only higher in injured drivers than in the general driving population, but the levels were also much higher in the first group. [9] Moreover, without quantitative bioanalysis the graded legislative BAC of 0.2, 0.5 and 0.8 g/L could not have been applied.

Although we obtained few data in our study, for illicit drugs different concentrations were seen in injured drivers versus the general population of drivers. [9] Graded risks are observed for several drug classes, [10,11] yet more data based on reliable quantitative research should be gathered in order to have a better view on the possibility of establishing risk thresholds for these drug classes.

While our data on ***THC*** showed no significant difference in concentration between controls (general driving population) and cases (seriously injured drivers), the case-control study conducted by Drummer et al. [12] concluded that THC positive drivers had a significantly higher likelihood of being culpable in fatal crashes than drug-free drivers. For drivers with blood THC concentrations of 5 ng/mL or higher, a higher statistically significant difference was found. This discrepancy between our study and the one of Drummer is due to the nature of the drug clearance. In fatally injured drivers the metabolism of THC stops, meaning that the concentration of THC in the post-mortem blood samples is the concentration at the moment of the crash. Since THC is a rapidly cleared drug (see Figure 7.1), [13–15] and the median interval between accident and blood sampling of the seriously injured drivers was 1.6 hours in our study, the detected concentration in blood was considerably lower than at the moment of the accident.

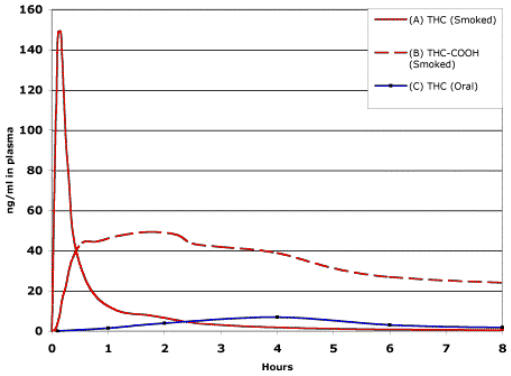

Figure 7.3. Plasma levels of THC and THCCOOH by time after intake. [13,14]

Although no significant difference in THC concentrations was found, Kuypers et al. [10] demonstrated a concentration-dependent crash risk for THC positive drivers. The statistical model used revealed a blood THC concentration of 2 ng/mL as breaking point at which the risk of having an accident was significantly increased. This value was in agreement with the study of Laumon et al. [16] who evaluated the relative risk of being responsible for a fatal crash while driving under influence of cannabis. They concluded that there was an increased risk of responsibility at whole blood THC concentrations above 1 ng/mL.

In 2007, an international working group of experts on issues related to drug use and traffic safety, funded by pro-cannabis organisations (Marijuana Policy Project, Washington DC and Dr. Bronner's Magic soaps, Escondido, California), discussed developing of *per se* limits for cannabis based on epidemiologic and experimental data. The epidemiological evidence was found to be insufficient for deriving a risk-based per se limit but a consensus of a serum lower limit of 7-10 ng/mL was proposed, since this concentration is correlated with impairment comparable to that of a BAC of 0.5 g/L. [17]

The DRUID study concluded that the risk associated with cannabis use was similar to the risk when driving with a low alcohol concentration (between 0.1 g/L and 0.5 g/L), being slightly increased to about 1-3 times that of sober drivers. The proposed risk threshold based on experimental studies for THC equivalent to 0.5 g/L alcohol was 3.8 ng/mL serum. [18]

Our data reveal higher concentrations for ***central stimulants*** in injured drivers. Although cocaine concentrations appeared higher in the general driving population, concentrations of its major metabolite benzoylecgonine were higher in injured drivers, suggesting higher cocaine levels at an earlier time. The cocaine results were probably affected by the time delay between accident and sampling in the injured drivers, which was up to 3h with a median of 1.6h.

For central stimulants, contrasting study results have been described in the literature. While for instance in Norway the use of central stimulants was found to be associated with increased risk of traffic accidents [19] other studies have shown only a trend towards increased risk. [12,16,20–22] Effects of central stimulants are euphoria, increased vigilance, motor activation, reduced judgement

and increased willingness for taking risks. [23] At low doses amphetamine and methamphetamine have shown to improve psychomotor effects. [24–27] Reduced SDLP (standard deviation of lateral position) has been noted after administration of 75-100 mg MDMA. [28,29] Slight reduction in driving skills was seen during daytime after ingestion of 30 mg dextro-amphetamine, [30] while ingestion of 70 mg led to hallucinations (visual and auditory) and reduced control of bodily and cognitive functions. [31] However, these doses are associated to blood concentrations that are much lower than the detected levels of amphetamine in DUID cases. While Gustavsen et al. [32] suggested a dose/concentration-effect for amphetamine, another study did not reveal such a relationship. [33] The stimulant effects are observed after ingestion of single doses, and mostly in sleep-deprived persons. Repeated administration may lead to impairment characterised by drowsiness and inattention, which may take several days to normalize, [23,34] meaning that even at low concentrations, substantial impairment can be observed after intake of a stimulant drug.
This lack of evidence concerning the dose/concentration and effect relation in the literature resulted in the absence of limits for graded sanctions regarding central stimulants in Norway. [35]

Different concentration levels between both groups of drivers have been observed for certain ***medicinal drug*** classes. [9] Surprisingly, most of the concentrations of the medicinal drugs were in the therapeutic range or lower than the therapeutic range. Further study might be needed to determine the role of these sub-therapeutic concentrations in the causation of accidents. For hypnotic benzodiazepines for instance, sub-therapeutic concentrations may be observed the morning after an intake around bedtime the night before.
Tolerance should also be taken into consideration, for instance with benzodiazepines, for which impairment has been reported to decrease after a few weeks. [36]
If lower than therapeutic concentrations are measured, the risk might be underestimated, for instance Kuypers et al. [10] calculated odds ratios (OR) for BAC groups using the Belgian DRUID data: the OR for all BAC (> 0.1 g/L) was almost 7, while the OR for a BAC between 0.1 and 0.5 g/L was approximately 1 and the OR for a BAC ≥1.2 g/L increased to more than 76. Our data also show that DUI with higher than therapeutic concentrations of medicinal drugs is rare in Belgium. Orriols et al. [37] investigated the association between prescription medicines and the risk of road traffic crashes in France. Medicines were grouped according to the four risk levels of the French classification system (from 0 [no risk] to 3 [high risk]). The population attributable risk of fatal accidents associated to driving with class 2 & 3 medicinal drugs in France was 3.3%; this means that if all driving with class 2 & 3 medicines was eliminated, the number of fatal accidents would decrease with 3.3%.
Other studies [38–42] suggest a higher risk for benzodiazepines and opioids, but these reflect more the illegal use of these medicines where higher doses are taken irregularly, resulting in higher concentrations in blood and more variation in concentration than in chronic medicinal use.

The therapeutic ranges used in chapter 4 were based on the ‘drug concentration list’ of The International Association of Forensic Toxicologists (TIAFT, previously available at http://www.tiaft.org/toxic_values). [43] These ranges are a well-based estimation, but can depend on

individual parameters such as co-medication, disease status, tolerance, metabolism status, age and gender,….

In this study ***combinations*** of more than one psychoactive substance (alcohol, (il)licit drugs) were not taken into account. However, combinational use is known to result in an exponentially increased accident risk, meaning stricter regulations should be implemented for cases of combined consumption. [18]

Although cases and controls covered the same area, the distribution of the cases over the hospitals was not representative, since some hospitals gathered few samples, resulting in a possible selection bias, which cannot be totally ruled out when conducting a multi-centre study. [44,45] In Flanders the number of injured drivers was over-represented (81% of the cases, 57% of the controls), in Wallonia the controls were over-represented (16% of the cases, 39% of the controls). [44,46] Since the prevalence results in the roadside survey did not differ significant between the different regions, the effect of this selection bias was expected to be very small. [45] The periods of recruiting were also not identical, but assumed to be equal, and not leading to bias.

1.4. Comparison between self-report of cannabis use and toxicological analyses in a roadside survey

Chapter 5 described the comparison between self-reported use of cannabis and toxicological data in a group of roadside respondents. Were there discrepancies between self-report and analyses in blood or oral fluid and was this related to the time after intake?

When using self-reported data, the prevalence of cannabis use can be underestimated. [47–50] This was also seen in our general population of drivers. [51] By comparing self-reported 'time after intake' and quantitative results of THC and THCCOOH in the biological fluids, it was observed that this underestimation was most obvious for recent use; people tended to give a more desirable answer to the question 'when was the last time of use' by stating for instance >24h or unknown while in fact the results of the quantitative bioanalysis suggested <4h.

It is remarkable that for some volunteers who reported to have used cannabis within 1 to 4 hours, this could not be detected. This is noteworthy since one would expect that a person would rather conceal using cannabis than report its use while this was not the case. We assume that this inconsistency could arise from the fact that the sense of time of certain respondents was not correct. Some drivers might have estimated their time of last use as 3 hours while in fact it was more likely 6 hours, which could have been reflected in the analytical results. Another possible explanation is the use of low potency cannabis or just inhaling one puff.

Besides the use of cannabis, also the consumption of other (il)licit drugs and alcohol was asked for. No correlation between for instance the use of alcohol and the reporting of time of use of cannabis was studied (e.g. the fact of not remembering when to have smoked because being under influence of alcohol). Alcohol use might have had an influence on the reporting, but since only 2.4% of the respondents had an BAC ≥ 0.5 g/L (and only 0.6% ≥ 1.2 g/L), this is found to be negligible.

Other self-reporting studies, such as in patients treated for drug addiction and university students, [52–59] observed a high level of consistency between self-reported use and biological testing. This is in contrast to our data, but it has to be kept in mind that our setting at roadside cannot be compared to a setting of students or drug rehabilitation clinic. In the latter, fear of retribution or criminal sanctions is low and cooperation is part of the treatment in drug clinics. In addition, these populations had a higher a priori chance of cannabis use than the general driving population in our paper. It is also difficult to compare our results with these studies due to differences in toxicological analyses. Most studies used immunological urine testing. [53,54,57] Since the window of detection of THCCOOH in urine is longer then our parameters in oral fluid or blood, toxicological findings were different. One study using oral fluid in a population of emergency department patients treated self-report as gold standard, putting there is no conceivable motive for being untruthful. [55] This is in contrast to our study in which we consider the toxicological analyses as standard. Table 7.2 gives an overview of some other studies investigating the agreement between self-report and toxicological analyses regarding cannabis use.

Table 7.25. Other studies evaluating agreement between self-report and toxicological analyses

Study	Sensitivity (%)	Specificity (%)	Kappa	Population	Gold standard	Toxicological analyses
Neale et al. [52]	n.r.	n.r.	0.44	Individuals beginning a new episode of drug treatment	Toxicological analysis	Oral fluid
Basurto et al. [53]	92	90	0.67	University students	Toxicological analysis	Urine (immunological)
Mayet et al. [54]	86	95	n.r.	French armed forces	Toxicological analysis	Urine (immunological)
McDonald et al. [55]	21.1	n.r.	n.r.	Emergency department patients	Self-report	Oral fluid (immunological)
Nichols et al. [56]	n.r.	n.r.	0.59	HIV-infected youth and youth at risk for HIV infection	Toxicological analysis	Blood (immunological)
Marroun et al. [57]	36	99	0.77*	Pregnant women	Toxicological analysis	Urine (immunological)
Hjorthøj et al. [58]	96	72	n.r.	Clinical trial with patients with cannabis use disorder	Toxicological analysis	Blood (LC-MSMS)

n.r.: not registered, *Yule's Y (similar to Kappa)

The study of Basurto et al. [53] has also shown that although agreement for cannabis was good, the sensitivity for reporting cocaine use was much lower (57%). This could partly be explained by the low number of positive self-reports (n= 7) and secondly by the fact that within this study it was observed that the attitude toward cannabis was more favourable than towards cocaine. This implies that agreement of parameters based on self-report of cannabis are not representative for the concordance of self-report of other psychoactive substances.

Quantitative results allowed to estimate the time after intake for persons who reported no use, based on their positive bioanalysis results for THC and/or THCCOOH in blood. [51] Therefore, the prediction models published by Huestis et al. were used (See chapter 6). [60]

In addition to information of the respondents, a dataset regarding non-responders was kept, with similar data such as age, gender and the result of the breath alcohol analyser, to be able to investigate possible non-response bias. In total 3206 drivers refused to participate, resulting in a high non-response rate (52%). Age and gender were significantly differently distributed in the response and non-response group. This could have led to bias, although the magnitude of these differences was small, and mainly significant due to the large sample sizes. Comparing both groups regarding distribution by breath alcohol analyser results, resulted in no difference, showing alcohol use did not influence the participation rate. This and the fact that the results of our self-report are similar to the results of the Health Interview Survey (see Chapter 6) makes us conclude that non-response did not bias the results. [46] On the other hand, the observation in Chapter 2 that a higher number of positives was seen in the group of respondents who only provided an oral fluid sample, might suggest bias. [2]

1.5. Overall conclusion

DUID legislation requires quantitative analysis of biological fluids to obtain results of drug concentrations under, at or above a set cut-off. In addition, quantitative bioanalysis is also significantly important in epidemiological research on drugs and driving, such as on drug use and accident risks associated with psychoactive substances. Accident risks for example should also be assessed in relation to the concentration and not only the presence or absence of the psychoactive substance. Self-reported data and quantitative bioanalysis can be complementary in epidemiological research, the latter giving more accurate data on (recent) drug use, the former obtaining more demographical data and information on the circumstances and history of drug use (e.g. last time of intake, route of administration, frequency of use).

2. Impact of enforcement

Does enforcement have an impact on traffic safety? Are more people caught driving under influence? Did fewer accidents occur since the new legal approaches are implemented? Does the number of DUID cases analysed in the toxicological laboratories increase?

In this part, data regarding enforcement of countries that have changed their legislation in recent years are briefly discussed.

2.1. Belgium

In Belgium, no research on the impact of the new legislation on DUID has been performed yet. But every year the Belgian Institute for traffic safety publishes statistics concerning enforcement. In the latest edition of 2014, data from 2007 until 2013 are presented. [61]

The highest number of traffic violations for driving under the influence of alcohol was noted in 2010, since then a decline is observed (see Figure 7.2). [61] Data regarding the annual BOB-campaign (a campaign against alcohol in traffic) at the end of the year suggest that less people drive under the influence of alcohol. The number of conducted breath tests has almost doubled from the period end 2004 – beginning of 2005 to 2012-2013, while the percentage of positive drivers has declined from 4.3 to 2.9. [61] This decrease is also seen in the behaviour-monitoring study performed every 2-3 years by the Belgian Institute for Traffic Safety, during which > 10,000 respondents are being checked with breath analysis. [62] In 2003, the percentage of drivers testing positive was 3.1, in 2012 this decreased to 2.4%.

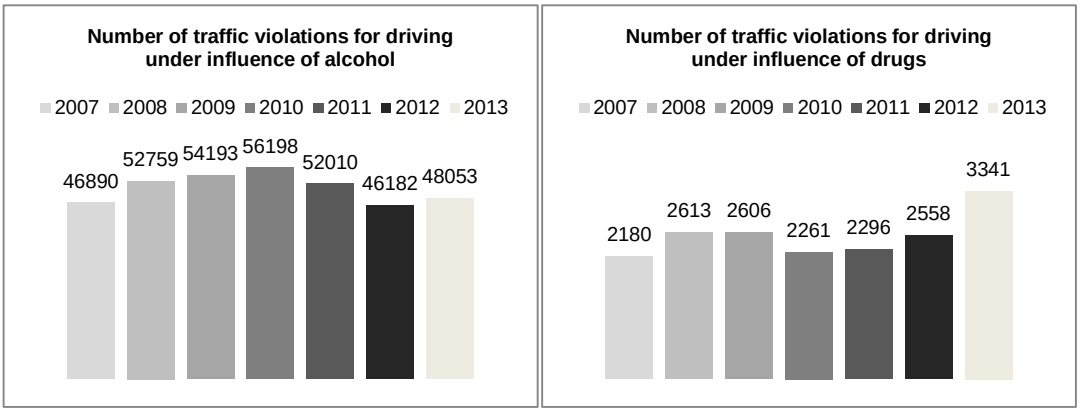

Figure 7.4. Number of traffic violations for driving under influence of alcohol or drugs (2007-2013). **[61]**

The number of traffic violations for driving under the influence of drugs has increased in the last few years to 3341 cases in 2013 (see Figure 7.2). [61] This can be the result of more people driving under influence or because of an increased probability of being caught and a higher number of screenings. Data described in chapter 3 of this dissertation suggest the latter. [5]

2.2. Other European countries

The change in legislation in ***France*** in 2008 allowing oral fluid screening has resulted in an increase in the number of roadside screenings. The number of confirmation analysis in blood has also increased with a factor of more than 5 between 2004 and 2010. [63]
At this moment, a feasibility study is being performed on the use of oral fluid in confirmation analysis. The collection device has been chosen as well as the biological markers and the analytical method is under development. [63]

The adaptation from impairment based to per se legislation in ***Denmark*** in 2007, has led to an increase in the number of confirmation analyses. Steentoft et al. [64] studied the prevalence of medication and illicit drugs among Danish drivers before and after 2007. The number of traffic cases investigated for substances other than ethanol ranged from 200 to 300 per year during the period from 1997 to 2006, but after the introduction of fixed concentration limits in 2007 a 5-fold increase was already seen in 2008.

In ***Norway***, Vindenes et al. [65] investigated the impact of implementation of legislative limits for drugs. The number of blood samples taken in suspected DUID cases increased by 20%. The number of samples with at least one drug above the *per se* limit corresponding to a BAC of 0.2 g/L increased by 17%, whereas the number of expert witness statements was reduced by half.
The researchers concluded also that the DUI legislation now signals that driving under the influence of psychoactive drugs is not compatible with safe driving, in the same way as for alcohol, and that it is likely that the new legislation leads to more convictions in court. The deterrent effect of the *per se* law would probably have been better if it had been widely published through information campaigns. [65]

Sweden's new zero-concentration limit for scheduled drugs in the blood of drivers has increased the number of DUID cases and successful convictions. However, the problem of drug-impaired driving is far from solved. People who drive after taking illicit drugs are mostly criminal elements in society who lack a valid driving permit and whose police records show many previous convictions for drunk and/or drugged driving as well as other deviant behaviour. Indeed, recidivism is close to 50–60% in these individuals so the zero-limit law has certainly not reduced DUID or functioned as a deterrent. However, the zero-limit law has brought more public and media attention to the problem of DUID and has made prosecution of cases a lot easier. The number of apprehended drivers increased from 600 to 700 annually in 1990, to around 7000 after introducing the zero-limit law. [66]

3. Future Perspectives

3.1. The role of 'new psychoactive substances' in the field of DUID

Should 'new psychoactive substances' (NPS) be included in legislation on driving under influence? In this part of the dissertation, the role of NPS in the field of DUID is discussed. What is the prevalence of NPS in general and driving population? What is their impact on driver impairment? And which studies regarding driving under influence of NPS have already been conducted?

3.1.1. Definition

New psychoactive substances are defined as new narcotic or psychotropic drugs that are not scheduled under the 1961 UN single convention on narcotic drugs or the 1971 UN convention on psychotropic substances. New does not always mean newly invented but also newly available or newly misused.

In 2014, 101 NPS were reported for the first time to the Early Warning System of the EMCDDA (European Monitoring Centre for Drugs and Drug Addiction) (see Figure 7.3). Currently more than 450 substances are monitored by the EMCDDA as NPS. In 2013, almost 47,000 seizures of NPS were reported amounting to more than 3.1 tonnes in Europe, a seven-fold increase compared to 2008. [67] This is still considerably lower than the amounts of seizures of classical drugs (herbal cannabis: 130 tonnes, cannabis resin: 460 tonnes, cocaine: 62.6 tonnes, heroin: 5.6 tonnes, amphetamine: 6.7 tonnes). [68]

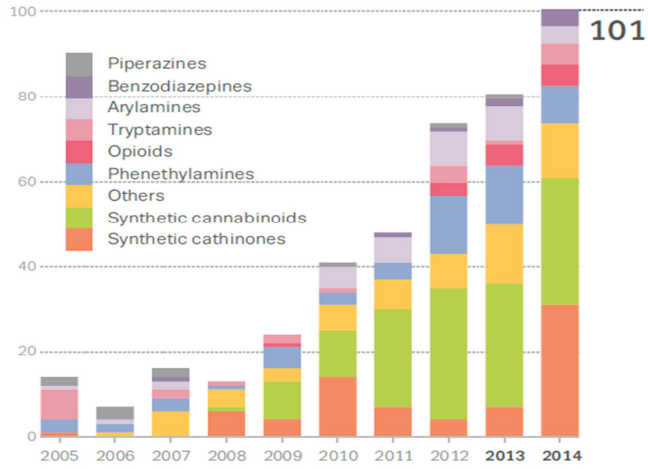

Figure 7.3. Number of newly reported NPS to the EU Early Warning System for the period 2005-2014. **[67]**

Synthetic cannabinoids and synthetic cathinones make up the largest groups of new psychoactive substances monitored by the EMCDDA; other groups include phenethylamines, opioids, tryptamines, benzodiazepines, arylalkylamines and piperazines. [67]

3.1.2. Prevalence of general use of NPS

Up to now, estimates on the prevalence of use of NPS in the general population are limited. Available information remains limited, collected in few countries, regarding specific substances and subpopulations. [8]

In the European Union (EU), the attitude of youth towards drugs is regularly examined by the Eurobarometer surveys. The last edition (Flash Eurobarometer 401) was carried out in 2014 in which more than 13,000 respondents aged 15-24 across the EU were interviewed by telephone. Eight percent of them reported to have used NPS, 1% in the last 30 days, 3% in the last 12 months and 4% more than a year ago. [7]

Information on NPS is gathered by case studies (e.g. persons admitted to emergency room), studying drugs found in party environment, wastewater analysis or by using questionnaires. The latter might for instance not be reliable since 1) people tend to underreport, 2) people don't know/remember names of new drugs and 3) mostly it is uncertain what people buy (e.g. bath salts can contain one time mephedrone, the other time with same packaging pentylone or combinations of different NPS).

3.1.3. The impact of NPS on driver impairment and subsequently road safety

In 2010, a review related to drug driving in the United Kingdom was published. [69] Since direct assessments of the impact of these drugs on driving ability are lacking, the investigators extrapolated the limited findings concerning effects of mephedrone, synthetic cannabinoids and benzylpiperazine (BZP) to make predictions as to what risks they might pose.

Mephedrone has both stimulant and euphoric/empathogenic properties similar to respectively amphetamines and MDMA. [69] Its effects on driver performance are likely to be similar to those of cocaine and amphetamines: increased risk-taking, loss of concentration and psychological impairment such as hallucinations or altered reality. [70] Mephedrone also causes visual impairment (e.g. tunnel vision), which consequently affects driving ability. [71]

Cannabis affects highly automated skills such as tracking and has some effect on perception, vigilance and coordination. [70] Since synthetic cannabinoids (SC's) have similar psychoactive properties but are more potent and with worse side effects, their effect on driving ability is assumed to be higher than regular cannabis. [69] Musshoff et al. [72] studied case reports on driving under influence of SC's and concluded that consumption can lead to performance deficits including centrally sedative effects and impairment of fine motor skills.

The stimulant and hallucinogenic properties of benzylpiperazine suggest it impairs performance similar to that observed after cocaine and MDMA intake: increased risk-taking, reduced concentration and distorted perception. [69]

Many (if not all) of the NPS are not included in standard screening panels in toxicological laboratories. This means that the biological sample of a driver suspected of impairment can return negative, due to the fact that the drug that was consumed was not detected. This creates a problem of enforcement, since proving a driver is impaired due to NPS is costly or sometimes not possible. [69]

3.1.4. Studies on NPS and DUID

In Belgium no data on the prevalence of NPS in plasma samples of apprehended drivers are available. In the Nordic countries several epidemiological studies on driving under influence of NPS have been conducted. Their DUID-cases were screened for defined NPS, from synthetic cannabinoids to synthetic cathinones or phenetylamines

In Norway, Tuv et al. [73] screened 726 drugged driving cases for detection of synthetic cannabinoids (SC). Approximately 2% was positive for one of the following NPS: AM-2201, JWH-018, RSC-4, JWH-122, JWH-081 and JWH-250. All 16 samples also tested positive for other drugs; in 75% of them regular cannabis was detected. Since other drugs were found in all the samples, the psychomotor impairment caused by the SCs was difficult to estimate.

Karinen et al. [74] have reported concentrations of APINACA, 5-APINACA, UR-144 and UR-144 degradant in whole blood samples collected from Norwegian DUID cases.

Tuv et al. [75] have investigated the frequency of methiopropamine (MPA) in DUID cases in Norway during a 12 week period. MPA was detected in ten DUID cases (0.8% of the cases), with other drugs concomitantly detected in all the cases. Two of the cases were traffic accidents.

Between October 2010 and May 2012, Kriiku et al. [76] analysed all serum samples from drivers apprehended on suspicion of DUID for desoxypipradol (2-DPMP) in Finland. Almost 2% of the cases were positive for this long-acting psychostimulant. Based on their findings, the researchers assume that 2-DPMP has an increasing detrimental impact on traffic safety in Finland.

Kriiku et al. [77] studied the presence of 3,4-methylenedioxypyrovalerone (MDPV) in blood samples for drivers suspected of DUID in Finland. Over a period of one year, MDPV was present in almost 6% of all confirmed DUID cases. In most cases amphetamine or benzodiazepines were also found. The researchers concluded that based on their findings, MDPV has a serious impact on traffic safety.

In the period 2009-2012, in Denmark a total of 15 forensic investigations, mainly DUID cases, involved 4-fluoroamphetamine; also 3 DUID cases with 2-fluoroamphetamine were reported. [78]

In addition, several case reports on driving under influence of a new psychoactive substance have been published. These case reports involve drivers suspected of driving under influence of drugs for which standard toxicological drug analyses revealed negative results. Additional screening was performed to identify possible NPS, in most cases synthetic cannabinoids. [72,79–83]

Regarding DUID-cases, questions could arise on false positive checklists (positive checklist, negative on-site test) or false positive screening results (positive on-site test, negative confirmation). Presently, there is little evidence of use of NPS in Belgium; in 2013-2014 only 10 Belgian cases regarding NPS in biological fluids (not DUID-cases) were reported to the Early Warning System of the EMCDDA. Screening of blood samples taken at the First Aid Post of the 'I Love Techno'-festivals (ILT) of 2013 and 2014 showed only one NBOMe and one GHB positive sample. [84] In addition, a project around 'Amnesty Bins' in several festivals in East-Flanders revealed almost no NPS (3 on a total of 146 samples, unpublished data). Both the Amnesty Bin and ILT projects also demonstrated that the classical drugs (cocaine, amphetamines, MDMA and cannabis) are most frequently used. All this leads us to conclude that NPS are not a priority for DUID-legislation in Belgium.

Besides the NPS, the question rises also to screen for other psychoactive substances such as GHB. In England a study revealed that only 5 out of 376 (= 1%) of suspected drugged drivers were positive for GHB in blood. [85] Recently, in the south of the Netherlands and the north of Belgium a trend of GHB impaired driving was observed. The Netherlands Forensic Institute investigated the prevalence of GHB in suspected impaired drivers by analysing the blood samples which were sent to the lab during the years 2009-2012. GHB was found in 669 out of the 3038 blood samples (= 22%) with concentrations ranging from 5.2 to 924 ng/mL (median 94 ng/mL). [86] In 27% of these cases no other illicit drug was detected. In Belgium, the toxicology laboratory of the national institute for criminalistics and criminology analyses GHB in blood samples of DUID cases if there is suspicion of GHB use mentioned in the police report. In 2014, out of 2680 DUID blood samples, 25 were also analysed for GHB, of which 48% was found positive. It should also be kept in mind that no screening test for GHB is available, and the endogenous concentrations of GHB in blood can hamper the interpretation.

3.2. Trends in alternative matrices

3.2.1. Oral fluid

Analysing oral fluid and interpreting the results is not always straightforward since it depends on the type of collection, which affects the final drug concentration within the total salivary composition. [87,88] While for instance matrix effects are observed due to the use of buffers in the collector, these stabilising buffers are necessary to guarantee good recovery from the collection path and analyte stability.

Salivary secretion is controlled by parasympathetic and sympathetic nerves, so several factors can have an influence such as circadian rhythm, hormonal changes, nutritional state, age, emotional state and type of stimulus. [89] Dilution might decrease drug concentrations as is increasing salivary pH. The ion trapping of basic analytes is highly influenced by salivary pH due to changes of the bicarbonate concentration in oral fluid. [89]

For urine and blood, quality control (QC) samples and proficiency testing (PT) are well established; this is not yet the case for oral fluid since no standardised protocol on sample collection is yet available. [90–92]

In addition, issues concerning interpretations can arise, such as passive versus active use, chronic versus occasional use or medicinal versus illicit use. For instance, passive contamination of oral fluid through passive smoking is a concern. [93,94] As the non-psychoactive THC-COOH is not present in cannabis smoke, the quantification of this metabolite can help to exclude passive contamination. [95] Since the Belgian legislation tackles recent use of cannabis and not passive contamination, it might be interesting to also measure THCCOOH when confirmation is based on oral fluid samples. However, it has to be kept in mind that in some cases when only THC is detected (and THCCOOH not), this does not mean that there was no active use as THCCOOH is often not detectable in occasional users. We assume that drivers that are passively exposed to cannabis smoke have a low risk of scoring positive on the checklist. In addition, studies reporting on passive cannabis smoke exposure, show oral fluid THC concentrations that are below the screening cut-off in oral fluid (=25 ng/mL). [93,94,96,97] In only two of these studies the concentrations were higher than de confirmation cut-off in oral fluid (= 10 ng/mL). [96,97] Moreover, the detection of THCCOOH is an analytical challenge since it is only present in low pg/mL in oral fluid. Some LC methods using derivatisation were developed, [98,99] while Scheidweiler et al. [100] developed a LC-MSMS method with an LOQ of 12 pg/mL.

Due to its lipophilic characteristic, residual concentrations of THC occur in chronic users resulting in possible interpretation errors. To determine recent use, analysis of cannabinol or cannabidiol in addition to THC is proposed. [101] However, the concentration of cannabidiol in oral fluid depends on the cannabidiol content of the cannabis strain that has been used and there is some concern about the stability of cannabinol in oral fluid. The use of the metabolite 11-OHTHC could be an option to differentiate active and recent use from chronic or passive use, but this component is only detectable in oral fluid in very low amounts and for a short time.

Other sampling techniques and analysing devices for detection of psychoactive substances in oral fluid are being developed. Stoykova and Atanasov performed a study using dry oral fluid spots. [102] They detected the presence of different psychoactive drugs such as methadone, (acetyl)codeine, heroin, cocaine, amphetamine, and methamphetamine. The presence of each compound detected in dry oral fluid was confirmed by liquid-liquid extraction and GCMS detection.

Analysing devices other than GC or LC based are also being investigated to develop a compact and easy-to-use test device for direct semi-quantitative drug testing in the field. For instance Hans et al. investigated the application of infrared attenuated total reflection (IR-ATR) spectroscopy for the detection of cocaine, its metabolites, diluents and masking agents in human saliva. [103]

3.2.2. Dried blood spots

The collection of a blood sample by means of a less invasive sampling technique would be a great improvement. Dried blood spot (DBS) sampling is a micro volume sampling technique where a small amount of whole blood (5 – 100 µL) is collected as a spot on a filter paper; capillary DBS can be collected simply by finger prick or heel prick. [104]

Dried blood spot sampling has many advantages compared to other conventional collection techniques. It is minimally invasive and collection can be done in a fast, simple and economic way, providing information on recent use of psychoactive substances. In addition, the time until sample collection can be reduced to a minimum when no medical staff is required, which is important in the context of DUID. [104]
DBS also improves analyte stability. Some substances rapidly degrade in whole blood, whereas the degradation process is reduced or minimized in DBS. This has been studied for benzodiazepines, zopiclone and cocaine. [105–107] This stabilising effect is also seen for 6-monoacetylmorphine, making the interpretation of possible heroin use more easy. [108] Furthermore, ex vivo formation of GHB or the alcohol biomarker phosphatidylethanol is not seen in DBS, in contrast to other biological fluids. [109,110]

This sampling technique also faces some challenges. Given the small volume that is available, highly sensitive detection techniques are often required, especially when only a few punches of the capillary blood are used for the analysis. The majority of methods reviewed by Sadones et al. show sufficient sensitivity to be relevant in the context of DUID, despite the fact that some compounds are clearly more challenging than others. [104] For instance, for cannabinoid quantitation the most promising method still required 20 µL DBS with a LLOQ of 1ng/mL (= Belgian legal cut-off). [111] Other challenges are the risk of contamination (for instance if the finger is not sufficiently decontamined), the acquisition of correctly obtained samples (can be checked by evaluating blank material), the influence of the site of punching, the blood volume spotted, and the haematocrit effect. [104] Even though blood-borne viruses like e.g. HIV and hepatitis C are inactivated on filter paper, the biohazard risk remains during collection. [112]

Another important factor in forensic cases is the ability to perform re-analysis or counter expertise; it should be possible to use spare DBS samples or the remaining parts of DBS. For this, satisfactory DBS quality – blood volume per DBS and the number of DBS per person – has to be established. [112]

While several reports have shown good correlations between concentrations determined in venous blood and in dried blood spots derived thereof, [113–115] reports describing the use of capillary DBS for DUID roadside testing are lacking. Sadones et al. [116] evaluated the correlation of GHB concentrations between capillary and venous DBS and concluded that blood obtained by finger prick was a valid alternative for venous blood. Nevertheless, equivalence between capillary and venous concentrations for other analytes will have to be demonstrated as well as the calculation of the legal cut-offs in whole blood instead of plasma. [104]

3.2.3. Volumetric absorptive microsampling

Volumetric absorptive microsampling (VAMS) is a novel sampling technique for the collection, transport, storage and analysis of biofluids that can be used to collect an accurate volume of blood (approximately 10µL), while reducing volumetric blood haematocrit bias. It contains a porous hydrophilic tip which, when touched to an aqueous fluid, absorbs the fluid into its internal volume through capillary action. The microsample is then dried and stored under ambient conditions until analysis which can be performed by a simple extraction procedure. [117] De Kesel et al. [118] evaluated the use of VAMS (for the compounds caffeine and paraxanthine) to overcome the haematocrit bias, a known concern when analysing DBS. The researchers concluded that VAMS assists in eliminating the effect of haematocrit. [118]

3.2.4. Exhaled breath

Recently, exhaled breath has been investigated as a matrix for detecting recent use of illicit drugs. Exhaled breath contains micro particles that can carry non-volatile compounds, including airway lining fluid from the lungs. If the lungs are contaminated with exogenous substances, they are trapped in the airway lining fluid and are distributed into the breath upon exhalation. [119]

Ellefsen et al. [119] developed a method for cocaine and its two major inactive metabolites benzoylecgonine and ecgonine methyl ester. They concluded that the presence of cocaine in breath after controlled intravenous administration indicates recent ingestion, but its absence does not exclude recent use.

Skoglund et al. [120] studied this new sampling technique in a clinical setting. Their main findings were that it is well tolerated by patients, it detects clinically relevant drug intake and that there is congruence between exhaled breath and plasma analytical findings. The exhaled breath technique can be an alternative when a recent drug intake has to be investigated. [120] Another study of Beck et al. [121] supports the use of exhaled breath as a new matrix in clinical toxicology.

Himes et al. [122] concluded that breath could be an alternative matrix for identifying recent driving under influence of cannabis, but sensitivity is limited due to a short detection window (<2h). A major limitation is the detection of occasional smokers shortly after smoking, since no occasional smoker produced a positive sample beyond 1 h after smoking. [122] Coucke et al. studied the relation of the breath concentration with some of the effects of tetrahydrocannabinol. [123] In this study the detection window after smoking one cannabis cigarette was at least three hours. THC was detectable in all samples, THCCOOH in none. The THC concentration in exhaled breath was related to the physiological changes in pulse rate and pupil diameter. Exhaled breath might be used to detect recent cannabis exposure.

More recently Stephanson et al. [124] developed a fully validated and robust screening method suitable for the routine measurement of drugs of abuse in exhaled breath with a simple procedure for specimen collection and sample preparation. The following analytes were covered: amphetamine,

methamphetamine, 6-acetylmorphine, morphine, cocaine, benzoylecgonine, diazepam, oxazepam and tetrahydrocannabinol.

A disadvantage of breath analyses for the detection of drugs of abuse is the requirement of very sensitive equipment and the difficulty of onsite screening for roadside testing.

A similar procedure as for breath alcohol testing might be an option in the future, since it is well-tolerated by people and there is no risk for adulteration or contamination. However, further research is needed on some particular issues e.g. transportation of bags of air and contamination of air with oral fluid particles.

3.2.5. Quality control and measurement uncertainty

After a method is validated, it is important to have an active control programme to continue ensuring the reliability of results. For this, quality control samples are used to guarantee an analytical run is acceptable, without problems during sample preparation or due to malfunction of an instrument. [125]

In addition to QC samples analysed in daily routine, proficiency testing is used to have an idea on how reproducible the results obtained by a certain method are. [125]

It is important that QC material is similar in composition to test samples, that it is stable, sufficiently homogeneous and available in sufficient quantities for analysis over a large time period. [126] Results of QC samples are plotted on a control chart or monitored by software and most laboratories use the 15/20 rule: 15% deviation is the accepted for QC samples with the exception near LOQ where 20% deviation is allowed. [125]

QC results are also important to calculate measurement uncertainty. Measurement uncertainty arises from the random and systematic effects inherent to measurement processes. It is the sum of all effects that cause variation in measurement results when the procedure is being performed correctly. Sources of measurement uncertainty can be: the sampling, sample composition, sample pre-treatment, reagents, laboratory equipment, instrumentation, environmental conditions in the lab. [126]

Measurement uncertainty is important when interpreting toxicological results. They have been estimated to be about 30% for drug-and-driving analysis, a logical percentage if 15% deviation for accuracy and precision are used. For post-mortem and hair analysis in forensic toxicology they are estimated to be respectively 40% and 50%. [125] One must pay attention to have an as low as possible measurement uncertainty when performing analysis in biological matrices.

When using alternative matrices, it is of great importance to develop a well-established QC and PT-scheme to increase quality assurance, which is currently not yet the case for oral fluid, DBS or breath analyses.

3.3. Trends in analytical technology

As mentioned in Chapter 1, more and more LC-HR/MS methods are developed and used in forensic toxicology. With HR/MS it is possible to detect more components with one method. For instance Concheiro et al. [127] have developed a method to simultaneously determine 40 novel psychoactive stimulants in urine with LC-HR/MS. The acquisition in full scan and data dependent MS2 mode allows flexibility to include additional NPS with a minimal validation. [127] Analysing a sample in full-scan with TOF gives the opportunity to search retrospectively for a certain new psychoactive substance at a later date. In addition, HR/MS methods could in combination with structure-based searches facilitate the identification of potential cross-reacting drugs or metabolites in immunoassays. Marin et al. [128] investigated false-positive amphetamine and MDMA urine immunoassays with a TOF mass spectrometer and an *in silico* structure search to generate a library of compounds known to have crossreactivity. HR/MS methods also have the advantage to analyse the glucuronide forms of certain substances such as THCCOOH, which could give extra information on recent use. Hädener et al. [129] concluded that the THCCOOH-glucuronide concentration in whole blood could complement free THCCOOH levels to distinguish between heavy (≥ 10 joints/month) and occasional (≤ 1 joint/week) cannabis users.

3.4. Further research

While comparing the legal approaches for DUID, it became clear that there are no studies on the impact of enforcement in Belgium. Data on the total number of on-site screenings performed are not available. A more detailed study on this matter could be an asset to see whether legislation has contributed to traffic safety e.g. has reduced the number of (fatal) accidents. To answer these questions, longitudinal studies are needed to gather information on positive samples for killed and injured drivers. Up till now no studies on killed drivers have been conducted.

How enforcement procedures can pay off, is part of an economic study which was outside the scope of this thesis, but could give valuable information, referring to the publication of Veisten et al. [130] based on work done within DRUID. [131] The final conclusion of this cost-benefit analysis was that increased drug driving enforcement based on roadside oral fluid screening is potentially beneficial, particularly for countries that currently have a low enforcement level. However, if the government decides to decrease drink-driving enforcement for the sake of financing increased drug driving enforcement (for a given budget), the net benefits of police enforcement will decrease (assuming that drink-driving will increase), since drink-driving is still a much bigger issue.

Further research on the false positives for opiates and amphetamines should be conducted, as well as on the role of NPS in DUID cases. To obtain these data, a retrospective analysis of plasma samples could be performed by screening for a large range of different drugs.

A future comparison will be made between confirmation in plasma and oral fluid, with both datasets linked to oral fluid on-site screening.

Further study on the role of (sub-)therapeutic concentrations of medicinal drugs should be performed, to investigate the possibility of setting impairment levels for common psychoactive medicines.

More research should be made, with more data, to see if the models built by Huestis et al. [60] could be used to predict time of cannabis intake in DUID cases and to see if roadside data could be used to predict prevalence in the general population, adding other psychoactive substances and also looking at prescription/pharmacy data. While case-control studies and prevalence studies in subgroups of drivers were performed in Belgium, conducting pharmacoepidemiological studies by linking police/legal data and medical data related to accidents have not yet been performed. An in depth analysis of the Belgian Institute for Trafic Safety showed that this lack of studies is related to privacy issues. At the time of the project ROPS, a recommendation was made that for every accident toxicological analyses should be performed to construct a database for statistical data processing. [132] However, in practice, this recommendation is not followed, partly due to the fear that the insurance would no longer want to cover costs in case of a positive toxicological result and because of the difficulty of covering the analysis costs.

Within DRUID, a pharmacoepidemiological study was performed in the Netherlands, to assess the association between traffic accident risk and psychotropic medication exposure by means of a case-control study. The data were gathered from three sources: pharmacy prescription data, police traffic accident data, and driving license data. A positive association was found between the risk of having a traffic accident and the exposure to at least one psychotropic medication. This association was found to be higher in combination therapy users. The highest risk groups were new users (although the association was not statistically significant), intermediate and long half-life benzodiazepine users (the association was statistically significant only for hypnotic intermediate half-life users), female users (the association was statistically significant only for hypnotic, antidepressant, and SSRI users), and young/middle-aged users (the association was statistically significant only for anxiolytic, antidepressant, and SSRIs users). [133]

The target set by the European Commission of halving the road deaths by 2010, was not met; by the end of 2010 a 43% reduction was reached. The Commission proposed to maintain the objective of halving the number of road deaths in the European Union (EU) by 2020, the aim is a maximum of 15,500 traffic fatalities. In 2014 the number of EU fatalities had dropped to 25 700. [134]

Although enforcement and public awareness campaigns are well established, some people are still driving under the influence of alcohol and other psychoactive substances. Does the future of traffic safety maybe lie in improving car security? Cars are already equipped with features in the first stage of ‘automatic driving’ such as lane control, automatic breaking, several airbags, sensors that warn a driver for a nearby obstacle, self-parking,... .

Building in alcohol interlock devices might also improve safety. Carter et al. [135] concluded that it is likely a cost-effective primary prevention policy that will substantially reduce alcohol-involved crash fatalities and injuries, especially among young vulnerable drivers and Radun et al. [136] concluded that they are an effective preventive measure against drunk driving when installed in the vehicles of convicted drunk drivers. Yet implementation is very costly mainly due to monitoring costs. Similar devices that could detect the presence of other psychoactive substances might be a subject for discussion and future research.

And what about fully automated vehicles (driverless cars)? Although this seems initially a perfect solution, some issues still need to be dealt with. What if suddenly a dangerous situation arises? What if suddenly a child crosses the road? In theory the car can warn his passenger but if that person is under influence of a psychoactive substance, his alertness is reduced. [137,138]
The cars are also not able to operate at a high level of safety in all weather conditions. Heavy rain can damage the sensors, calling into question what role the driver might have to play in the event the technology fails. If for instance traffic signals that the cars rely on are disrupted, there's no accounting for human traffic signals. A driverless car also relies completely on GPS-signals for mapping of roads and signals, which are in some cases not error-free. Thus there is still a long way before driverless cars are fully ready for prime time … or rush hour. [138]

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ADDENDUM

ABBREVIATIONS

2-DPMP	2-diphenylmethylpiperidine
6-MAM	6-monoacetylmorphine, 6-acetylmorphine
Amu	Atomic mass unit
APINACA	N-(1-adamantyl)-1-pentyl-1H-indazole-3-carboxamide
ATS	Amphetamine type stimulants
BAC	Blood alcohol concentration
BIVV	Belgisch instituut voor verkeersveiligheid
BZP	Benzylpiperazine
CI	Confidence interval
CYP	Cytochrome P450
DBS	Dried blood spot
DIA	Data-independent acquisition
DRUID	Driving under the influence of drugs, alcohol and medicines
DUI	Driving under the influence
DUID	Driving under the influence of drugs
EDDP	2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
ELISA	Enzyme-linked immunosorbent assay
EMCDDA	European monitoring centre for drugs and drug addiction
EU	European Union
FN	False negative
FP	False positive
GC-MS	Gas chromatography – mass spectrometry
GHB	Gamma-hydroxybutyric acid
GPS	Global positioning system
HHMA	3,4-dihydroxymethamphetamine
HIS	Health interview survey
HMMA	3-hydroxy-4-methoxymethamphetamine
HR/MS	High resolution mass spectrometry
ILT	I Love Techno
IMMORTAL	Impaired motorists, methods of roadside testing and assessment for licensing
IR-ATR	Infrared-attenuated total reflection spectroscopy
JWH	John W. Huffman, creator of the JWH cannabinoids
LC-MS	Liquid chromatography – mass spectrometry
LLE	Liquid liquid extraction
LLOQ	Lower limit of quantification
LOD	Limit of detection
LOQ	Limit of quantification
LSD	Lysergic acid diethylamide
M6G	Morphine-6-glucuronide

MAIS	Maximum abbreviated injury score
MBDB	*N*-Methyl-1,3-benzodioxolylbutanamine
MDA	3,4-Methylenedioxyamphetamine
MDEA	Methylenedioxyethylamphetamine
MDMA	3,4-Methylenedioxymethamphetamine
MDPV	3,4-Methylenedioxypyrovalerone
MPA	Methiopropamine
n.a.	Not applicable
NPS	New psychoactive substance
n.r.	Not registered
OF	Oral fluid
OH-THC	11-hydroxy-Δ9-tetrahydrocannabinol
OR	Odds ratio
PMA	Para-methoxyamphetamine
PMMA	Para-methoxymethamphetamine
PP	Protein precipitation
PPV	Positive predictive value
PT	Proficiency testing
QC	Quality control
RSC-4	(4-methoxyphenyl)(1-pentyl-1H-indol-3-yl)methanone
SAMHSA	Substance abuse and mental health services administration
SC	Synthetic cannabinoid
SDLP	Standard deviation of the lateral position
SPE	Solid phase extraction
THC	Δ9-tetrahydrocannabinol
THCCOOH	11-nor-9-carboxy-Δ9-tetrahydrocannabinol
TIAFT	The international association of forensic toxicologists
TN	True negative
TOF	Time-of-flight
TP	True positive
UK	The United Kingdom
UPLC	Ultra-high performance liquid chromatography
UPLC-MSMS	Ultra-high performance liquid chromatography – tandem mass spectrometer
UR-144	(1-pentylindol-3-yl)-(2,2,3,3-tetramethylcyclopropyl)methanone
VAMS	Volumetric absorptive micro sampling
WP	Work package

CURRICULUM VITAE

Gertrude (Trudy) Van der Linden was born on 15th February 1982 in Eeklo. She studied Biomedical Science at Ghent University and graduated in July 2008. In September 2008 she worked as lab assistant at the Institute for agriculture and fishery research (ILVO: Instituut voor Landbouw- en Visserij Onderzoek) in Melle. In November 2008 she joined the Department of Clinical Chemistry, Microbiology and Immunology, where she worked at the European project Driving Under the Influence of Drugs, alcohol and medicines (DRUID). From April 2010 to March 2011 she worked at the Scientific Institute for Public Health as coordinator of the 'Belgian Early Warning System on Drugs'. In April 2012 she joined the laboratory for drug analyses of the National Institute for Criminalistics and Criminology as a judicial expert. In June 2013 she enrolled as a PhD candidate at the Faculty of Medicine and Health Sciences of Ghent University. The results of her PhD project are presented in this thesis.

LIST OF PUBLICATIONS – INTERNATIONAL

Van der Linden T, Wille SM, Ramírez-Fernandez M, Verstraete AG, Samyn N. Roadside drug testing: comparison of two legal approaches in Belgium. Forensic Sci Int. 2015 Apr;249:148-55. doi: 10.1016/j.forsciint.2015.01.034. Epub 2015 Feb 4.

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LIST OF PUBLICATIONS – DRUID DELIVERABLES

DRUID-Deliverable 2.2.3 ‘Prevalence of alcohol and other psychoactive substances in drivers in traffic in general in 13 member states’

DRUID-Deliverable 2.2.5 ‘Prevalence of alcohol and other psychoactive substances in injured and killed drivers.’

DRUID-Deliverable 2.3.5. ‘Relative accident risk for impaired drivers based on case control studies in seven member states’

DRUID-Deliverable 3.2.1 ‘Protocol of workshop on drug driving detection by means of oral fluid screening’

DRUID-Deliverable 3.2.2 ‘Analytical evaluation of oral fluid screening devices and preceding selection procedures’

DRUID-Deliverable 4.2.1 ‘Establishment of Criteria for a European categorisation system for medicines and driving’

DRUID-Deliverable 4.3.1 ‘Establishment of framework for classification/categorization and labeling of medicinal drugs and driving’

DRUID-Deliverable 4.4.1 ‘Classification of medicinal drugs and driving: Co-ordination and synthesis report’

DRUID-Deliverable 7.2.1 ‘Recommendations for improving medical guidelines for assessing fitness to drive in patients who use psychotropic medicines

DRUID-Deliverable 7.2.2 ‘Guidelines and professional standards: report and CD with example of ICT supported protocols for prescribing and dispensing of medicines affecting driving performance, and for informing patients who use other psychoactive substances than medicines’

DRUID-Deliverable 7.3.1 ‘Prototypes of booklets, posters, messages for risk communication including a script for a TV-clip’

DRUID-Deliverable 7.4.1 ‘Training manual for physicians and pharmacists on medicinal drugs and driving’

DRUID-Deliverable 7.4.2 ‘Report on the implementation, evaluation and new technologies of practice guidelines and information materials’

DRUID-Deliverable 7.4.3 ‘DRUID outcomes and risk communication to young drivers’

Druid deliverables are consultable on the website www.druid-project.eu

LIST OF PUBLICATIONS – OTHER

T. Van der Linden, S. M. Wille, M. del Mar Ramirez-Fernandez, A. G. Verstraete, N. Samyn. Comparison of narcotic drug concentrations in plasma after roadside testing in urine or oral fluid in Belgium. Accepted for presentation at the 53rd Annual Meeting of TIAFT, Florence, 2015.

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